School of Science
Department of Environment and Agriculture

Regulation of fruit softening, colour development and quality in controlled atmosphere stored mango

Maria Fransisca Sumual

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due the acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: _____

Date: _____

Dedication

This is dedicated to my family ***Kaligis - Sumual***

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List of symbols and abbreviations

%	percent
°C	degree Celcius
Δ	changes in
µg	microgram(s)
µl	microlitre(s)
µmol	micromole(s)
1-MCP	1-methylcyclopropene
AA	ascorbic acid
AAE	ascorbic acid equivalent
ACC	1-aminoclyclopropene-1-carboxylic acid
ACO	1-aminocyclopropane-carboxylic acid oxidase
ADC	arginine decarboxylase
ADH	alcohol dehydrogenase
AIH	agmatine iminohydrolase
ANOVA	analysis of variance
AOA	aminooxiacetic acid
APX	ascorbate peroxidase
ARG	arginine
ATP	adenosine triphosphate
C_2H_4	ethylene
C_4H_6	cycloalkene
CA	controlled atmosphere
CAT	catalase
CHA	cyclohexylamine
CI	chilling injury
CO_2	carbon dioxide
dcSAM	decarboxylated SAM
DFMA	difluoromethylarginine
DFMO	difluoromethylornithine
DPPH	2,2-diphynyl-1-picrylhydrazyl
EC	enzyme commission number

FAO	Food and Agriculture Organisation
FRAP	ferric reducing antioxidant potential
GAE	gallic acid equivalent
JA	jasmonic acid
LOX	lipoxygenase
M	molar
MAP	modified atmosphere packaging
MET	methionine
MGBG	methylglyoxal-bis(guanylhydrazone)
MJ	methyl jasmonate
mM	millimolar
Mt	metric tonne(s)
MTA	methylthioadenosine
NA	normal atmosphere
NCP	*N*-carbamoylputrescine
NCPase	NCP amidohydrolase
O_2	oxygen
ODC	ornithine decarboxylase
OPDA	12-oxo-phytodienoic acid
ORAC	oxygen radical absorbance capacity
ORN	ornithine
PAL	phenylalanine ammonia-lyase
PAs	polyamines
PE	pectin esterase
PG	polygalacturonase
PPO	polyphenol oxidase
Put	putrescine
SAM	*S*-adenosylmethionine
SAMDC	SAM decarboxylase
SAMsyn	SAM synthase
SOD	superoxide dismutase
Spd	spermidine
Spdsyn	spermidine synthase

Spm	spermine
Spmsyn	spermine synthase
TA	titratable acidity
TCA	tricarboxylic acid
TE	trolox equivalent
TSS	total soluble solids
UK	United Kingdom
US	United States

Abstract

Postharvest storage, transport and distribution of mango fruit have limitations due to its high perishability and susceptibility to chilling injury and postharvest diseases. Controlled atmospheres (CA) in supplementation with low temperature storage have been known to delay fruit ripening, alleviate chilling injury and control postharvest diseases of the fruit. The sea-freight of mangoes from Australia to distant markets in CA containers is a commercial practice with some constraints. However, the optimum CA conditions and storage temperature (13°C) are not capable of maintaining desired fruit texture, colour and taste during transport and distribution period. There is a scope for enhancing the efficacy of CA by combining with other postharvest treatments aimed at delaying fruit softening. The objective of this study was to enhance the efficacy of CA to improve postharvest quality and stability of mango fruit through synergistic effects anticipated from postharvest treatments with methyl jasmonate (MJ), putrescine (Put) and 1-methylcyclopropene (1-MCP).

The atmospheres comprised of 3% O_2 with 4, 5 or 6% CO_2 were more effective in maintaining fruit firmness than normal air (NA) until 4 weeks of storage at 13°C. During 4 weeks of CA storage, mango fruit retained green skin colour, although there was some reduction in chlorophylls and increased carotenoids contents both in skin and pulp. Sugars and organic acids also accumulated during the CA storage period. During ripening, post-CA storage fruit showed higher activity of *exo*-PG than those fruit retrieved from NA storage in contrast to the activity of *endo*-PG and PE. Ripe CA-stored fruit also showed slower colour development and chlorophylls degradation, while retaining high glucose and organic acids concentration.

Methyl jasmonate combined with CA storage reduced the activity of *exo*-PG at 4 and 6 weeks stoarge, increased skin carotenoids at 4 weeks, but not 2 or 6 weeks, total sugars at 4 and 6 weeks, ascorbic acid at week 4, but decreased at 6 weeks, and reduced total acids, whereas having no significant effect on the other measured quality parameters compared to CA storage. The suppressed activities of *exo*-PG

enzymes correlated negatively, whereas *endo*-PG and PE correlated positively with firmness during 2 to 4 weeks of storage.

Postharvest application of Put combined with CA storage resulted in better firmness and springiness after 4 weeks of storage compared to CA storage alone. Firmness was positively correlated to *endo*-PG and PE, and negatively correlated to *exo*-PG. Put-treated fruit maintained its carotenoids content in fruit skin at low level resulted in retardation of colour and chlorophylls degradation during 4 weeks of CA storage. Sugars to acids ratio was higher in Put-treated fruit stored in CA within 4 to 6 weeks compared to untreated fruit under CA storage. All CA-stored fruit pulp accumulated lower carotenoids compared to NA-stored ones.

The combination of pre-storage application of 1-MCP and CA storage was not more effective as compared to CA alone in retarding fruit softening and activity of fruit softening enzymes, and improving skin colour rating, skin pigments, total sugars, and total antioxidants within 5 weeks of storage. During ripening, the combination of 1-MCP treatment and CA storage showed an improvement similar to CA storage alone in overall quality of fruit following 3 weeks of storage, and prolonged storage to 5 weeks resulted in low quality of ripe fruit.

In conclusion, CA storage comprising of 3% O_2 + 5% CO_2 prolonged storage life of 'Kensington Pride' mango. The application of MJ, Put, and 1-MCP resulted in improved fruit quality during 3 to 4 weeks of CA storage to a limited extent.

Chapter 1
General introduction

Mango (*Mangifera indica* L.) fruit is extolled worldwide for its pleasant aroma, delicious taste and attractive colour. Cultivation of mango has been known since the ancient times, spreading in Southern Asia, especially India, Burma, and Malay Archipelago. In the last few centuries, mango has spread in different part of the world, including Australia. The Food and Agriculture Organization recorded that at least 94 countries are commercially producing mango. The world production of mango was estimated at 43.3 million tonnes from 5.4 million ha area worth US$ 43633.40 million (FAOSTAT, 2015). The policy of the world free trade promotes the volume of the mango exported to different places, especially to the non-producing countries such as European and North American countries. Recently, the estimation of mango production in Australia was 41,911 tonnes in 2014 (ABS, 2015) with the value of US$106.6 million (FAOSTAT, 2015). In 2014/2015 season, the mango fruit exported from Australia was about 7,000 tonnes. This exported volume increased about 30% from its previous season (Anonymous, 2015).

Mango is a climacteric fruit which ripens within 7 to 9 days when harvested at mature green stage and stored under ambient temperature (21°C). The acceleration of respiration and ethylene production induces faster ripening process leading to shorter shelf life (Mattoo and Modi, 1969). The highly perishable nature of this fruit results in high postharvest losses. Estimated mango losses in postharvest handling were amount to 8.6 million tonnes worth US$ 335.2 million per year (Kader, 2005b).

Postharvest handling techniques have been developed in order to manage the product quality after harvest. Modification in storage conditions including storage atmosphere composition, temperature, and duration of storage (Lalel et al., 2001), ripening conditions, and postharvest treatments including physical (Jacobi et al., 2001a) and chemical treatments (Malik, 2003) have been reported to improve postharvest shelf-life and fruit quality of mango. Sea freight containers with CA at low temperature are being used to ship mango from Northern Territory and Western

Australia to London and Dubai. Poor skin colour development, high acidity, and fruit softening were encountered as the major postharvest problems associated with sea freight (Anonymous, 2002). During 2009/2010 season, Australian mangoes were shipped to Japan and China in CA containers with more consistent fruit quality on arrival (AMIA., 2012). Earlier studies on CA storage focused on maximising the storage life (Singh et al., 1937) and improving flavour and aroma production (Dang, 2007; Lalel, 2002). However, the development of off-flavour (Beaudry, 1999; Bender and Brecht, 2000; Noomhorm and Tiasuwan, 1995), poor colour (Bender et al., 2000c; Lalel et al., 2006) and texture deterioration (Lalel et al., 2003a) have been reported as the major degrading effects in mangoes during CA storage and ripening. It was therefore hypothesized that the changes in texture, colour and other quality parameters may be influenced by storage condition, duration, and pre-storage treatments.

The exogenous application of methyl jasmonate, a plant regulator (Creelman and Mullet, 1995), has been reported to activate the ethylene-forming enzyme, 1-aminocyclopropane-carboxylic acid oxidase (ACO) in tomato fruit (Czapski and Saniewski, 1992) and stimulate ripening in mango (Lalel et al., 2003g). Several studies on the role of methyl jasmonate in fruit ripening have been conducted (Ayala-Zavala et al., 2005; Fan et al., 1998a; Gonzalez-Aguilar et al., 2004; Khan and Singh, 2007a), including mangoes (Gonzalez-Aguilar et al., 2001; Lalel et al., 2003g) during normal course of ripening. However, information regarding the role of methyl jasmonate in maintaining mango fruit quality under controlled atmosphere storage is lacking.

The exogenous application of polyamines, including putrescine, has been reported to delay ripening and senescence of various fruit and vegetables (Arava et al., 2000; Khan and Singh, 2007b; Kramer et al., 1991; Martínez-Romero et al., 2002; Mirdehghan et al., 2007; Perez-Vicente et al., 2001; Purwoko et al., 1998; Torrigiani et al., 2004; Winer and Apelbaum, 1986), including 'Kensington Pride' mango (Malik and Singh, 2006; Malik et al., 2003). Putrescine treatment has improved texture in plums (Khan and Singh, 2007b; Perez-Vicente et al., 2002), delayed colour development in apricot (Martínez-Romero et al., 2002; Martínez-Romero et al., 2001; Torrigiani et al., 2004) and improved quality of 'Kesington Pride' mango (Malik and Singh, 2006) during storage at ambient atmosphere. There

is little information regarding the influence of putrescine treatment on the storage life and quality of mango fruit kept under controlled atmosphere, which warrants investigation.

Pre-storage application of 1-methylcyclopropene (1-MCP) has been shown to inhibit ethylene action in plants (Sisler and Serek, 1997), hence delaying ripening and ripening-related changes. The application of 1-MCP on various cultivars of mango fruit has been previously reported (Hofman et al., 2001; Hojo et al., 2006; Jiang and Joyce, 2000; Krishnamurthy et al., 1971; Lalel et al., 2003f; Lima et al., 2006; Osuna-García et al., 2009; Pandey and Singh, 2007; Penchaiya et al., 2006; Wang et al., 2006). The application of 1-MCP to 'Kensington Pride' mango has also been resulted in delayed ripening (Razzaq et al., 2015). The exogenous application of 1-MCP followed by modified atmosphere packaging (MAP) on 'Tommy Atkins' mango (Cocozza et al., 2004a; Cocozza et al., 2004b), and CA storage of hot-water-treated 'Kent' mango (Lima et al., 2006) has been reported to reduce softening during storage. Information on the influence of 1-MCP treatment in combination with CA condition on quality improvement during storage and ripening of 'Kensington Pride' mango is limited and inconclusive.

An understanding of these factors may lead to the development of postharvest strategies to maximise the beneficial effects of CA storage on fruit quality in 'Kensington Pride' mango fruit. Therefore, this research was conducted with the following objectives:

1. To investigate the effects of different compositions of CA storage on fruit firmness, colour development and quality during storage and ripening.
2. To elucidate the role of methyl jasmonate together with CA storage in improving the physico-chemical quality of mango fruit.
3. To evaluate the effects of pre-CA storage application of putrescine in modulating fruit firmness, softening enzymes activities and other quality changes in mango fruit.
4. To assess the role of 1-MCP treatment together with CA storage in regulating fruit softening, colour development and overall quality during storage and ripening of mango fruit.

Chapter 2

General literature review

2.1. Introduction

Mango (*Mangifera indica* L.) is the second largest tropical fruit crop in the world with a total production of 43.3 million tonnes harvested on 5.44 million hectares in 2013 (FAOSTAT, 2015). The major producing regions are mostly in the tropics, with India contributing about 42 % of the world production followed by China (10%), Thailand (7%) and Indonesia (5%) (Table 2.1). Contribution of Asia to the world mango production was more than 80%. However, mango is also exported by some countries that are not major producers (Figure 2.1). Mexico is the largest mango exporter followed by India and Thailand, accounting for 23%, 17%, and 16% respectively of the fresh mango fruit world market in 2012.

In addition to the tropical countries as the major producers, some regions outside that boundary such as Israel, California, and Australia also produce mango fruit (Crane et al., 1997). Australian mango plantations are located in Queensland, Western Australia, Northern Territory, and New South Wales. The mango industry in Western Australia provides fresh mango fruit from October to April every year (Anonymous, 2005; Anonymous, 2015; Johnson and Parr, 2005) and it is the second most important tropical fruit in the state after banana. In the year of 2013-2014, Western Australia mango production was 2,588 tonnes (ABS., 2015) which equals to 6% of total Australian mango production (FAOSTAT, 2015).

Mango fruit has a unique aroma and delicious taste. It is also a rich source of vitamin A, minerals and antioxidants (Kondo et al., 2005a). Increased demand for fresh mango worldwide is shown by an increasing import quantity of this fruit. Total world mango fruit import was 1,100,000 tonnes in 2012. The United States and European Union, contributed to about 33% and 35%, respectively, of the total imports (FAOSTAT, 2015). In Australia, more than 65,100 tonnes fresh mango fruit were marketed during the mango season in 2014/2015 (Anonymous, 2015).

Table 2.1. Mango producing countries and their contribution to the world production in 2013

Country	Area harvested (ha)	Production (tonnes)	% Contribution
India	2,500,000	18,002,000	41.6
China	465,000	4,450,000	10.3
Thailand	380,000	3,141,950	7.3
Indonesia	196,000	2,058,607	4.8
Mexico	198,883	1,901,871	4.4
Pakistan	171,289	1,658,562	3.8
Brazil	70,372	1,163,000	2.7
Bangladesh	124,000	950,000	2.2
Nigeria	115,000	795,000	2.0
Egypt	91,770	834,543	1.9
Philippines	196,248	831,026	1.9
Vietnam	78,156	705,865	1.6
Kenya	47,154	582,907	1.3
Peru	32,707	461,214	1.1
Tanzania	35,103	450,041	1.0
Cuba	40,678	389,576	0.9
Yemen	25,842	381,783	0.9
Madagascar	47,000	305,000	0.7
Colombia	23,390	270,826	0.6
Australia	9,300	40,797	0.1
Others	578,482	3,870,502	8.9
World (Total)	5,441,374	43,300,070	100

Source: FAOSTAT (2015).

The leading commercial mango cultivar grown in Australia is ‘Kensington Pride’. This cultivar has bright orange-yellow skin with a red-pink blush in areas exposed to the sun. The flesh of the fruit is orange, thick, practically fibreless, juicy, and rich in flavour. Due to its appearance, fruit is liked by the European and North American consumers (Morton, 1987). High production and low consumption of mango in Australia are the driving forces behind the Australian mango exports.

Australian mango production is contributing lesser amount to the world production (Table 2.1); however, the export during 2014/2015 season reached the amount of 7,000 tonnes. It was 30% increase from previous season in Australian mango export (Anonymous, 2015). The prospect of expanding the overseas market, however, poses a challenge for the sea cargo system to develop a better storage environment and postharvest technology, since mango fruit is a highly perishable horticultural commodity.

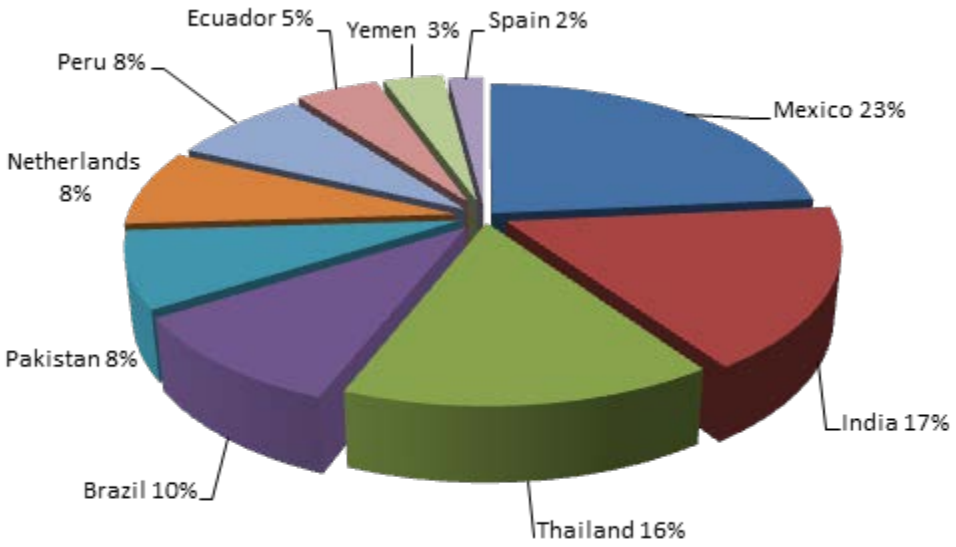

Figure 2.1. The percentage of mango export by major countries during 2012 (FAOSTAT, 2015).

Marketability of fruit depends mostly on consumer acceptability, which is mainly due to the sensory characteristics that can be easily detected such as flavour, colour, size, shape, and texture (Eskin, 1990; Tucker, 1993). Nevertheless, the awareness of consumers on the nutritional value of produce is also important (Tucker, 1993). All these quality attributes are altered during ripening of detached fruit and the changes differ in accordance with the environmental factors such as temperature, humidity (Gast, 2005; Paull, 1999), air composition (Thompson, 1996), maturity stage at harvest and mango variety (Singh and Singh, 2012).

The mango industry has been facing the major challenge in marketing of the fresh produce, i.e., inconsistent fruit quality on arrival at the markets, thus reducing consumer satisfaction. The inconsistency in fruit quality mainly varies due to

maturity level of fruit on display, which is affected during handling, transportation and other stages of the supply chain. This review will present a general idea of research work concerning this issue, specifically the role of low oxygen (O_2) and high carbon dioxide (CO_2) atmosphere, which will provide a better understanding to overcome the problem.

2.2. Mango fruit ripening

Fruit ripening process is considered as a set of biochemical reactions under strict genetic control toward senescence. The ripening process is initiated from the final stage of fruit development to the beginning of senescence. The crucial factor in determining the rate of metabolism in detached produce is maturity stage at harvest (Seymour et al., 1990; Singh and Singh, 2012), which can affect the shelf life and quality of fruit after storage (Siriphanich, 1998; Tucker, 1993). Fruit harvested either earlier or later than their optimum maturity stage result in lower quality (Seymour et al., 1990; Singh and Singh, 2012). The ripening process of mango fruit is reported to take place within 4 to 8 days after harvest depending on cultivar, maturity stage and environmental conditions (Gowda and Huddar, 2000; Nassur, et al., 2015; Singh and Singh, 2012).

Harvesting fruit before the optimum maturity stage increases the sensitivity to mechanical injury (Chonhenchob and Singh, 2003), chilling injury, and further influences the development of skin colour during ripening (Ledger, 1995; Medlicott et al., 1988). In contrast, delaying harvest results in internal breakdown of mango fruit (Lee et al., 1998) and short storage life (Seymour et al., 1990), but better aroma (Bender et al., 2000a). Mango fruit harvested at optimum maturity stage are suitable for postharvest heat treatments such as vapour-heat and hot water, which are required to meet quarantine requirements of certain importing countries (Jacobi et al., 2001b; Jacobi and Wong, 1992).

Mango fruit, as other fleshy fruit, ripening is a process in which the biochemistry and physiology alterations occur to influence the appearance, texture, flavour, and aroma. Increase in respiration rate and ethylene synthesis is considered as the core of biochemical reactions involved in the ripening process of climacteric fruit (Eskin, 1990; Tucker, 1993) that leads to the changes in carbohydrates, organic

acids, lipids, phenolics, and volatile compounds (Gomez-Lim, 1993; Singh and Singh, 2012).

Environmental conditions, temperature and relative humidity, are amongst the external factors accountable for the fruit ripening process and the durability of storage life. The optimum temperature for mango fruit ripening varies among cultivars (Table 2.2). The deviation of temperature from the optimum range results in unfavourable mango fruit quality. Poor mango fruit quality is due to retention of higher acid content and lower sugars accumulation, reduced carotenoids synthesis both in pulp and skin, and poor flavour and aroma development during ripening (Lalel, 2002; Medlicott et al., 1986a; O'Hare, 1995; Thomas, 1975; Vazquez-Salinas and Lakshminarayana, 1985). Mango fruit ripened under normal condition exhibits transformation of physical and chemical characteristics, including texture and chlorophyll degradation, carotenoid formation, starch hydrolysis, reduction of organic acids and improved flavour and aroma (Gomez-Lim, 1997; Ito et al., 1997; Kulkamp et al., 2004).

Table 2.2. Optimum ripening temperature of different mango cultivars

Mango cultivars	Optimum ripening temperature (°C)	Reference
'Alphonso'	25	Thomas (1975)
'Tommy Atkins'	22	Medlicott et al. (1986a)
'Heiden', 'Irwin',' Keitt', 'Kent'	20-22	Vazquez-Salinas and Lakshminarayana (1985)
'Kensington Pride'	20	O'Hare (1995); Lalel (2002)

2.2.1. Respiration

Respiration is the fundamental process in living organisms where stored organic materials are catalysed into simple end products with the release of energy (Kays, 1991). Carbohydrate is the major organic material present in the form of sucrose and starch in higher plants. During respiration, carbohydrates are oxidized to CO_2 and water, with a production of adenosine triphosphate (ATP) as energy stored within the plant cells (Eskin, 1990). This process needs O_2 in order to achieve

complete oxidation of carbohydrates. Respiration also can occur with less or without oxygen but leads to less degradation of carbohydrates and lower energy production. The later is called anaerobic respiration (Eskin, 1990) or fermentative metabolism (Vigneault et al., 2003; Watkins and Zhang, 1998). However, the production of carbon dioxide increases during fermentative metabolism under certain low level of oxygen named anaerobic compensation point (ACP) or respiration quotient breakpoint (Chervin et al., 1996).

Climacteric and non-climacteric fruit are distinguished according to their respiration rate and pattern (Tucker, 1993). Climacteric fruit such as mango shows a characteristic peak of respiratory activity during ripening. The increase in respiration rate of mango fruit represents normal ripening process (Singh and Singh, 2012). In contrast, non-climacteric fruit displays a gradual decline in their respiration during ripening (Eskin, 1990; Tucker, 1993).

The pattern of respiration rates of mango fruit are stimulated by both internal and external factors such as cultivars, maturity stage, ethylene, and postharvest handling including storage temperature and atmosphere composition, disease and high temperature treatments (Ducamp-Collin, 2001). Mango fruit shows moderate respiration rate (Kader, 1992) which is quantitatively measured as CO_2 produced during ripening under certain temperature (Paull and Ching, 2005; Thompson, 1996). Respiration rate of mango fruit at 5°C is 10 – 20 mg CO_2 $kg^{-1}hr^{-1}$ (Kader, 1992; Paull and Ching, 2005; Thompson, 1996) and increases as the temperature increases (Paull and Ching, 2005; Thompson, 1996). 'Kensington', 'Irwin' and 'Neelum' mangoes stored at temperatures ranging from 21 – 29°C showed the respiratory climacteric after 2 to 6 days of ripening period (Ito et al., 1997; Kulkamp et al., 2004; Lalel et al., 2003d).

The nature of mango fruit to ripe within a short period of time reduces the storage life, which has led researchers to regulate the respiration by interrupting the respiration with less interference on the quality (Singh and Singh, 2012; Tucker, 1993). Usually, climacteric fruit are harvested at immature or mature green stage and ripened in controlled conditions of temperature and humidity. The respiration rate of mango fruit can be successfully retarded by low temperature storage (Veloz et al., 1977). However, immature fruit can fail to ripen properly (Tucker, 1993) and can be more susceptible to chilling injury when stored at temperature below 13°C

(Leticia, 2003). The development of acceptable flavour and nutritional quality of fruit stored in low temperature can also be obstructed by low temperature storage (Singh and Singh, 2012).

The modification of O_2 and/or CO_2 rates during storage has been used to reduce respiration rate, hence improving the storage life of agricultural produce (Thompson, 1996; Singh, 2015). Storage of mango fruit under modified and controlled atmosphere has proven to extend the shelf life (Abdulah and Basiouny, 2000; Lalel et al., 2001; McLauchlan and Barker, 1992; Singh and Zaharah, 2015; Yahia, 1998).

2.2.2. Ethylene

Ethylene (C_2H_4), a plant hormone, plays an important role in many phases of plant growth and development. In postharvest of horticultural produce, hastening of senescence and shorter shelf life are the detrimental effects of C_2H_4. However, faster and more uniform ripening of products is an advantageous effect, commercially exploited to regulate fresh fruit market demand-supply (Reid, 1992). Ethylene can increase the respiration rate and alter the cellular structure, result in deterioration of quality attributes of the stored produce (Kays and Paull, 2004). Normal ripening process of climacteric fruit requires a burst of C_2H_4 production which is concomitant with the respiration peak (Alexander and Grierson, 2002). A large increase in respiration rate and C_2H_4 production of the climacteric fruit coincides with the ripening process. Storage conditions may induce or slow down these changes hence induce or slow down the deterioration process (Eskin, 1990; Thompson, 1996).

Methionine is an amino acid that starts C_2H_4 biosynthesis via the pathway that includes other key compounds as intermediate named S-adenosyl-methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC). ACC synthase, the enzyme controls the rate of biosynthesis in the pathway, is activated by pyridoxal phosphate. However, some compounds known as C_2H_4 inhibitors are aminoethoxyvinyl glycine (AVG) and aminooxyacetic acid (AOA) which require pyridoxal phosphate (Reid, 1992). Inhibition of ethylene-forming enzyme, ACC oxidase (Gomez-Lim, 1997), can also be induced by cobalt ion and low O_2 concentration which in turn restrain C_2H_4 production (Reid, 1992). ACC oxidase inhibitor in unripe mango fruit was discovered as acetaldehyde (Burdon et al., 1996)

which may be accumulated in fruit under oxygen stress. Mathooko (1996a) revealed that C_2H_4 biosynthesis is mainly affected by CO_2 and suggested three points where CO_2 can regulate C_2H_4 biosynthesis i.e., during conversion of SAM to ACC which is catalyzed by ACC synthase, conversion of ACC to C_2H_4 catalyzed by ACC oxidase, and the metabolism of ACC to 1-malonylamino cyclopropane-1-carboxylic acid (MACC). However its effect differs depending on the commodity, cultivar, and maturity stage (Watkins and Zhang, 1998).

Mango has been classified as a horticultural commodity with moderate C_2H_4 production rate, i. e., 1.0 to 10.0 $\mu l \cdot kg^{-1}h^{-1}$ at 20°C (Kader, 1992). During ripening of 'Tommy Atkins' mango, maximum C_2H_4 production occurred on day 6 together with the maximum CO_2 released (Medlicott et al., 1986a). In unripe 'Keitt' mango fruit, the production of C_2H_4 is less than 1 $nl \cdot g^{-1}h^{-1}$ (Burdon et al., 1996). In pre-climacteric mango fruit, C_2H_4 production is low but then increases noticeably during the climacteric phase (Gomez-Lim, 1997).

Ethylene, both endogenous and exogenous application, exhibits some effects on the fruit quality. Poor textural property was monitored during the ripening of 'Dashehari' mango fruit treated with high dose of ethrel (Pal, 1998). Green mature 'Kensington Pride' mango treated with ethylene at 15°C for 72 h resulted in early loss of firmness but less chlorophyll degradation and high acidity (Nguyen and McConchie, 2002). 'Karuthacolomban' mango cultivar had less weight loss and minimum changes in physicochemical properties when packed in low-density polyethelene (LDPE) bag with C_2H_4 and CO_2 scavengers. The ripening of this cultivar was delayed up to 21 days (Illeperuma and Jayasuriya, 2002).

2.2.3. Colour

Peel colour is an important factor determining fruit acceptability. Gradual change in fruit peel colour occurs during ripening together with other changes. These changes take place directly after the climacteric rise in respiration (Eskin, 1990). During ripening of most mangoes, the dark green colour of fruit peel changes to olive-green, sometimes red, orange-yellow or yellow depending on cultivar (Mitra and Baldwin, 1997; Singh and Singh, 2012). Pigments responsible for fruit colour are chlorophyll, carotenes including xantophylls and xantophyll esters, and anthocyanin (Lakshminarayana, 1980; Lizada, 1993). Chloroplasts containing

chlorophyll in the peel are transformed into chromoplasts containing red and yellow carotenoid pigments during ripening (Mitra and Baldwin, 1997). Several processes have been suspected to mediate the chlorophyll degradation. These processes are triggered through the action of chlorophyllase, enzymatic oxidation, or photodegradation. Oxygen, temperature and light have been implicated as environmental factors that can affect the postharvest synthesis of carotenoids (Eskin, 1990; Goodwin and Goad, 1970; Mitra and Baldwin, 1997).

Chlorophyll degradation takes place rapidly during ripening in some mango cultivars such as 'Keitt', 'Tommy Atkins', and Kensington Pride' mango (Medlicott et al., 1986a; Medlicott and Thompson, 1985; O'Hare, 1995). In accordance to the degradation of chlorophyll, carotenoids can be synthesized both in skin and pulp of mango fruit through the ripening process (Medlicott and Thompson, 1985; O'Hare, 1995; Singh and Singh, 2012). Carotenoids, the major pigments in ripe mango, are present in various forms. Typical profiles of carotenoids in the ripe mango were reported to vary due to cultivar differences (Cano and de Acos, 1994; John et al., 1970; Mercadante and Rodriquez-Amaya, 1998; Vásques-Caicedo et al., 2006). The major carotenoid in fully ripe 'Badami' mango was β-carotene that accounted for 50.64% of total carotenoids (John et al., 1970). All-*trans*-violaxanthin comprised 38% - 47% of total carotenoids in 'Keitt' mango (Mercadante and Rodriquez-Amaya, 1998; Mercadante et al., 1997). The *trans*- isomers of β-carotene were the most predominant carotenoids in mango fruit cv. 'Tommy Atkins' (Vásques-Caicedo et al., 2006). In 'Palmer' mango fruit, total carotenoids content was 2.63 mg·100 g^{-1} with *β*-carotene accounting for 25% of the total (Ribeiro et al., 2007).

Temperature during storage and ripening of mango fruit was suggested to play an important role in carotenoids metabolism (Medlicott et al., 1986b; Singh et al., 2013; Thomas, 1975; Vazquez-Salinas and Lakshminarayana, 1985). 'Alphonso' mango fruit stored continuously in low temperature synthesis less carotenoid compared to fruit kept at room temperature (Medlicott et al., 1986b; Thomas, 1975). Moreover, low temperature storage of 'Kent' mango fruit prior to ripening at room temperature resulted in low carotenoids content (Veloz et al., 1977). Carotenoid content in 'Tommy Atkins' mango fruit pulp at temperature more than 22°C was 3.5-4.0 mg·100 g^{-1}, but adverse result was found when the fruit stored in lower temperature (Medlicott et al., 1986b). It was suggested that the duration of cold

storage also affected the synthesis of carotenoids in ripe mango fruit (Medlicott et al., 1986b; Thomas, 1975; Vazquez-Salinas and Lakshminarayana, 1985). Other external factors that were reported to have an influence on the carotenoids synthesis were C_2H_4 treatments on 'Kensington Pride' mango (Nguyen and McConchie, 2002) and atmosphere composition (McLauchlan and Barker, 1992). 'Kensington Pride' treated with C_2H_4 ripened at 15°C had more green colour compared to that at 20°C and higher. Longer exposure to C_2H_4 also retarded the chlorophyll breakdown on the skin of ripe mango fruit (Nguyen and McConchie, 2002). Ripe 'Keitt' mango derived from hot climate accumulated more β-carotene than those from moderate climate part of Brazil (Mercadante and Rodriquez-Amaya, 1998; Mercadante et al., 1997).

2.2.4. Texture

Textural softening in fruit is associated with ripening which determines fruit quality and consumers acceptance. The modes of textural changes in mango fruit are cultivar dependent (Selvaraj and Kumar, 1989). Medlicott et al. (1986a) reported a complete change of texture in 'Tommy Atkins' 9-day after harvest. Polysaccharides in the cell wall such as pectin, cellulose, and hemicellulose undergo depolymerisation, de-esterification, and solubilisation due to the action of hydrolytic enzymes (Selvaraj and Kumar, 1989; Singh et al., 2013; Singh and Singh, 2012; Singh and Zaharah, 2015). Pectolytic enzymes alter the size and the nature of pectic polymers in fruit ripening process. Solubility of cell wall pectic polymers increases during mango fruit ripening, thus promoting fruit softening (Al-Haq and Sugiyama, 2004; Lazan et al., 1986; Roe and Bruemmer, 1981; Tandon and Kalra, 1984). The pectin solubility starts from the inner mesocarp tissues of mango fruit followed by the outer part tissue; consequently the inner mesocarp of ripe mango is softer than the outer tissue layer (Lazan et al., 1993; Mitcham and McDonald, 1992).

Cell wall hydrolytic enzymes responsible for the depolymerisation and the modification of polymers in mango fruit are polygalacturonases (PG), pectinesterases (PE, EC 3.1.1.11), *endo*-1,4-β-D-Glucanases, β-galactosidases (β-Gal, EC 3.2.1.23), pectate lyases (PL, EC 4.2.2.2), cellulases (EC 3.2.1.4), galactanases (EC 3.2.1.145), arabinanases and xylanases (EC 3.2.1.8); those enzymes collaborate to reduce the molecular size of pectic polymers and then to increase the solubility of pectic substances within the cell wall to bring about fruit softening (Abu-Sarra and Abu-

Goukh, 1992; Ali et al., 1990; Ali et al., 1995; Ali et al., 2004; Ashraf et al., 1981; Chourasia et al., 2006; Chourasia et al., 2008; Lazan et al., 1993; Lazan et al., 1986; Mitcham and McDonald, 1992; Prasanna et al., 2003; Prasanna et al., 2005; Roe and Bruemmer, 1981).

The degradation of cell wall at α-(1-4)-linked galacturonic acid residues is performed by polygalacturonase (PG) (Lazan et al., 1993). This enzyme exists in two forms, i.e., *exo*-PG (EC 3.2.1.67) and *endo*-PG (EC 3.2.1.15). The activity of PG has been reported to appear at the onset of ripening and increase through the ripening process of mango fruit (Abu-Sarra and Abu-Goukh, 1992; Ali et al., 2004; Chaimanee, 1992; Lazan et al., 1986; Mitcham and McDonald, 1992; Prasanna et al., 2003; Roe and Bruemmer, 1981). Both enzyme, *exo*- and *endo*-PG hydrolyse the polymers of α(1-4)-galacturonic acid, however *exo*-PG specifically act at terminal end while *endo*-PG hydrolyses randomly (Abu-Sarra and Abu-Goukh, 1992; Lazan et al., 1993; Lazan et al., 1986; Suntornwat et al., 2000). The e*xo*-PG activity is more pronounced during ripening of 'Nam Dok Mai' (Suntornwat et al., 2000) compared to *endo*-PG, and it is highly related to ripening (Chaimanee et al., 2000). The activity of *exo*-PG increases as the ripening of mango fruit proceeds (Abu-Sarra and Abu-Goukh, 1992; Ali et al., 2004; Mitcham and McDonald, 1992; Prasanna et al., 2003; Roe and Bruemmer, 1981). In mango cv. 'Keitt', the activity of PG was reported to correlate to the loss of firmness (Roe and Bruemmer, 1981). Similarly, increased activity of *endo*-PG and cellulase were significantly correlated with the reduction in firmness of 'Kitchener', 'Dr Knight' and 'Abu-Samaka' mango cultivars during ripening (Abu-Sarra and Abu-Goukh, 1992). Moreover, Mitcham and McDonald (1992) reported that the PG activity is higher in the inner mesocarp than in outer tissue, which causes the difference of softening rate in mango fruit. However, some contradictions in the correlation between PG activity and mango fruit softening have been acknowledged by Abu-Sarra and Abu-Goukh (1992) and Lazan et al. (1986).

Pectinesterase (PE) is responsible for the de-esterification of methyl group in galacturonic acid chain during mango fruit ripening, hence increasing water-solubility nature of pectin. It has been reported that the activity of this enzyme is lower in fruit pulp compared with skin (Abu-Sarra and Abu-Goukh, 1992; Ali et al., 1990; Roe and Bruemmer, 1981; Tahir and Malik, 1977). The irregular activity of

this enzyme has been reported, i.e. in some cultivars it declines and in others it shows a constant trend during fruit ripening (Ali et al., 2004; El-Zoghbi, 1994; Prasanna et al., 2003; Roe and Bruemmer, 1981). During fruit ripening, PE activity declined continuously in 'Samar Bahisht Chaunsa' (Razzaq, et. Al., 2014), 'Kensington Pride' (Razzaq et al., 2015), 'Kitchener' and 'Dr Knight' mangoes, but increased in 'Abu-Samaka' mango (Abu-Sarra and Abu-Goukh, 1992). In contrast, PE activity increased during fruit ripening in Pakistan mango cv. 'Anwar Ratual', 'Langra', 'Chaunsa', and 'Dusehri' (Ashraf et al., 1981) and in African mango (*Irvingia gabonnsis* var. gabonensis) (Aina and Oladunjoye, 1993).

In 'Harumanis' mango, the increase in activity of β-D-galactosidase (E.C. 3.2.1.23) was parallel to the decrease in firmness during ripening (Ali et al., 1995). Besides pectin degradation, increased activities of some carbohydrate hydrolases such as mannanase (E.C. 3.2.1.78) and galactosidase (E.C. 3.2.1.23) were also detected during ripening of 'Alphonso' mango that caused textural softening (Bhagyalakshmi et al., 2002). The degree of solubility of pectin or non-pectin components in cell wall was suggested to be the factor that affected the texture of 'Kent' mango (Banjongsinsiri et al., 2004).

Temperature showed indifferent effects on texture during ripening of mango fruit. The texture of 'Tommy Atkins' mango fruit was not significantly changed during storage under temperature range between 22 and 37°C (Medlicott et al., 1986b). Furthermore, texture of 'Kent' and 'Haden' ripened at 35°C and 37°C respectively, was firmer than those ripened at lower temperatures (Medlicott et al., 1988). Under other extremely low temperature conditions, the firmness of tree ripe 'Irwin' mango stored at 5°C can be sustained up to 20 days (Shivashankara et al., 2004). A large reduction in cellulose, hemicellulose and pectin content during ripening of 'Alphonso' mango stored at 27°C has been reported by Bhagyalakshmi et al. (2002), which coincided with fruit softening. Softening of 'Kensington Pride' mango occurred more rapidly than other changes when fruit were treated with high C_2H_4 concentration at 15°C for 72 hours (Nguyen and McConchie, 2002). Similarly, 'Neelum' mango fruit dipped in ethrel also showed a decline in firmness (Kulkamp et al., 2004).

2.2.5. Compositional changes

Fruit ripening and senescence are oxidative processes which occur within the cells involving enzymes and hormones. Alterations in physical appearance, eating quality and nutritional values of fruit are correlated to biochemical compounds and secondary metabolites, which are accumulated or lost during fruit ripening due to the activation of biochemical and molecular-levelled reactions (Bouzayen et al., 2010).

Carbohydrates are well known as the major components which play an important role in fruit physiology. Starch is accumulated in mango fruit during maturation (Fuchs et al., 1980). Metabolic fluctuation during ripening causes drastic changes in carbohydrates and organic acids. The starch which is stored in chloroplasts is hydrolysed into sugars in mango fruit at the ripening stage (Ito et al., 1997; Kumar et al., 1994; Medlicott et al., 1986b; Selvaraj and Kumar, 1989). The conversion of complex carbohydrates to simple sugars has been reported in various mango cultivars. Starch content in 'Alphonso' mango decreased from 14% in the immature fruit to 0.3% in ripe fruit (Ito et al., 1997). Decrease in starch content has also been reported in 'Haden' and 'Irwin' mangoes (Castrillo et al., 1992; Ito et al., 1997).

The development of sweetness in ripe fruit is due to the accumulation of soluble sugars as the result of the degradation of polysaccharides. The total soluble solids (TSS) of mango fruit increase tremendously from harvest to ripe stage. Total sugars and soluble sugars contents in mango fruit increase during ripening under normal atmosphere condition as have been reported by Sharaf et al. (1989). TSS increased 8% in 'Alphonso' (Thomas, 1975), 6.7% in 'Keitt' (Medlicott and Thompson, 1985), and 7.8% in 'Kensington Pride' mangoes (O'Hare, 1995). In general, total sugars in ripe mango fruit is in the range between 10 – 20%. The variations in total sugars content and the sugars composition depend on cultivar and the ripening stage of the fruit (Hubbart et al., 1991; Krishnamurthy et al., 1971; Lakshminarayana, 1973; Litz, 2009; Medlicott and Thompson, 1985; Shashirekha and Patwardhan, 1976). 'Tommy Atkins' mangoes ripened at temperature below 20°C were high in glucose compared to those ripened at 22 and 27°C (Medlicott et al., 1986b). The influence of temperature on sugar composition was also reported in 'Alphonso' mangoes, in which glucose and fructose were higher when fruit were stored at low temperatures (Thomas, 1975). Major increase in soluble sugar, mainly

reducing sugar, and large reduction of starch, cellulose, hemicellulose and pectin content during ripening of 'Alphonso' mango stored at 27°C were observed by Bhagyalakshmi et al. (2002). Sucrose has been reported as the predominant sugar in ripe mango fruit, followed by fructose and glucose (Castrillo et al., 1992; Gil et al., 2000; Hubbart et al., 1991; Medlicott and Thompson, 1985; Vazquez-Salinas and Lakshminarayana, 1985; Yashoda et al., 2006). The composition of sugars in ripe 'Keitt' mango was dominated by sucrose, followed by fructose and glucose (57%, 28%, and 15%, respectively) as has been reported by Medlicott and Thompson (1985). Gradual reduction of glucose and fructose accompanied by continuous accumulation of sucrose during fruit ripening has also been reported in 'Haden', 'Irwin', 'Kent' and 'Keitt' (Vazquez-Salinas and Lakshminarayana, 1985). The sucrose in ripe mango fruit increases 1.3 fold from the unripe mango (Gil et al., 2000; Medlicott and Tompson, 1984).

The mechanisms for the increase in total sugars are identified as starch degradation into simple sugars and sucrose synthesis during fruit ripening. Starch is degraded into soluble sugars catalysed by amylase (EC 3.2.1.1) during fruit ripening (Fuchs et al., 1980; Mattoo and Modi, 1969; Tandon and Kalra, 1984). The sucrose synthesis during mango fruit ripening is denoted by increase in activity of sucrose phosphate synthase (EC 2.4.1.14) and sucrose synthase (EC 2.4.1.13), although the later is lower (Castrillo et al., 1992; Hubbart et al., 1991; Kumar et al., 1994). The activity of sucrose synthase is higher compared to the activity of invertases (EC 3.2.1.26), therefore the fructose and glucose are lower than sucrose in ripe fruit (Castrillo et al., 1992).

Organic acids contribute to acidic properties due to their carboxyl groups. The ratio of sugars and acids play an important role in flavour perception by consumers (Malundo et al., 2001; Singh and Singh, 2012). Considerable losses of organic acids occur during ripening of mango fruit. In 'Tommy Atkins', acidity declines drastically by day 9 (Medlicott et al., 1986b). It has been reported that citric and malic acids are the principal organic acids in mature mango fruit while other organic acids such as tartaric, ascorbic, fumaric, succinic, shikimic, oxalic, and α-ketoglutaric acids are minors (Medlicott and Thompson, 1985; Sarker and Muhsi, 1981; Shashirekha and Patwardhan, 1976). In 'Badami' mangoes, citric acid represents the major organic acid whilst malic and succinic acids present in low

concentration (Shashirekha and Patwardhan, 1976). Citric acid is the major organic acid in unripe 'Tommy Atkins' mango fruit, and its amount decreases as ripening proceeds (Gil et al., 2000). In 'Irwin' mangoes, citric acid decreased after 20 days stored at 5°C regardless of its stage of maturity (Shivashankara et al., 2004). It has been reported that oxalic, citric, malic, pyruvic and succinic acids are presented at the detectable amount in 'Fazli' and tartaric in 'Zardalu' mangoes (Kumar et al., 1993). Ito et al. (1997) reported an increase of citric acid during developmental stages of 'Irwin' mango fruit, and decrease during ripening.

Biosynthesis of organic acids takes place through the tricarboxylic acid (TCA) cycle. The concentrations of α-ketoglutaric acid and pyruvic acids are at their maximum prior to the climacteric peak of 'Pairis' mangoes whereas the utmost level of aspartic and glutamic acids appears after 3 days of harvest (Krishnamurthy et al., 1971). Enzymatic transformations and oxidative decarboxylation reactions have been reported during three-quarter-ripe and ripe stages of 'Alphonso', Banganpalli', Dasheri', 'Fazli', 'Langra' and 'Suvarnarekha' mangoes (Selvaraj and Kumar, 1994). Baqui et al. (1974) reported that the concentration of malic dehydrogenase (EC 1.1.1.37) and succinic dehydrogenase (EC 1.3.5.1) increase at the beginning of ripening while citrate synthetase (EC 2.3.3.1) increases extensively during maturation of 'Alfonso' mangoes. The activity of malic enzyme achieved its maximum just after climacteric rise and decline during postclimacteric (Dubery et al., 1984). The synthesis of citric acid is catalysed by acid invertase with high activity at the initial stage of endocarp hardening, and decrease as the maturation and ripening proceeds. An extensive decrease in acidity is mostly due to the reduction of citric acid content in ripe fruit whilst the loss of malic acid is very small (Medlicott and Thompson, 1985).

Generally, aromatic compounds are integrated to consumer perception on fruit flavour. Mango aroma is formed by a mass of volatile compounds (Singh and Singh, 2012). Factors affecting aroma volatile production in mango fruit such as cultivar, maturity at harvest, ripening conditions, chilling injury, postharvest treatments, growth regulators including ethylene and jasmonates, and storage conditions have been reported (Lalel, 2002; Lalel and Singh, 2004; Lalel et al., 2001; Lalel et al., 2003a; Lalel et al., 2003b; Lalel et al., 2003d; Lalel et al., 2003e; Lalel et al., 2003f, g; Lalel et al., 2004, 2006; Nair and Singh, 2003; Singh et al., 2004). The

effect of other factors such as rootstock (Dang, 2007), polyamines, hot water treatment (Dea et al., 2010), edible coatings (Dang et al., 2008a) and fungicide treatments (Dang et al., 2008b) have also been reported. In mango fruit, terpenes have been discovered to be the most abundant volatile compound among other major groups, followed by esters, ketones, and lactones (Singh and Singh, 2012). Previously, 30 volatile components were identified in mango fruit, including cis-ocimene, myrcene, limonene, esters, aldehydes and terpenes (Sharaf et al., 1989). More recently, aroma volatile compounds in 'Kensington Pride' mango have been identified as much as 61 compounds, of which 35 compounds were reported as new volatile compounds discovered in this cultivar. The aroma compounds in 'Kensington Pride' mango comprise 59% hydrocarbons and 20% esters group which are the most abundant volatile groups (Lalel et al., 2003a). Within the first 7 days of ripening, mango produced the major compound α-terpinolene, which was replaced by ethyl octanoate afterwards. The synthesis of major monoterpenes, except car-3-ene, increased during the first 3 to 4 days of ripening and decreased subsequently. The major sesquiterpenes were intensively synthesised at the early ripening stage. In accordance to the production of terpenes, the three major esters were also synthesised intensively during fruit ripening. It appeared that the synthesis of terpenes and esters were in mutual accord to the synthesis of ethylene and fatty acids, respectively (Lalel et al., 2003a).

Fatty acids, although available in very small quantities, play an important role in fruit aroma and flavour (Bandyopadhyay and Gholap, 1973; Lalel et al., 2003a). A decline in linoleic acid and an increase in linolenic acid contents, together with equal distribution of palmitic and palmitoleic acids, are noticed in ripening of 'Alphonso' mangoes (Bandyopathyay and Gholap, 1973). They also reported that the ratio of palmitic acid to palmitoleic acid is in correlation with the aroma and flavour characteristics of 'Alphonso' mango. Palmitic and oleic acid have been identified as the major fatty acids in ripe mango (Sharaf et al., 1989). The total fatty acids content in ripe mango fruit is influenced by ripening conditions. Total fatty acids in ripe 'Kensington Pride' mango fruit increased as the ripening temperature increased to 30°C. All major fatty acids increased during the ripening process except palmitic, palmitoleic, and linolenic acids (Lalel et al., 2004). The concentration of glyceride increases as the changes in fatty acids take place in 'Alphonso' mango pulp

during ripening (Bandyopadhyay and Gholap, 1973). The production of esters contributing to aroma volatile in 'Kensington Pride' mango has been associated with increasing fatty acids (Lalel et al., 2003a).

Other compounds which undergo changes during fruit ripening are known as secondary metabolites. The secondary metabolites are organic compounds that are not engaged directly in regulating the growth or reproduction of organisms; instead they contribute to the aesthetic performance, flavour and plant self defence mechanism. Recently, increasing evidence indicates that many of these compounds are important in human health as they exhibit antioxidant activity and other pharmacological properties (Noratto et al., 2010). These compounds can be classified into different groups of chemicals such as phenolic compounds, carotenoids and vitamins such as vitamin C and E (Gonzalez-Aguilar et al., 2008). The principal antioxidants in mango fruit are carotenoids, ascorbic acid, and phenolics (Manthey and Perkins-Veazie, 2009).

Polyphenolic compounds in several cultivars of mango fruit have been characterized such as quercetin, kaempferol glycosides, gallic acid, m-gallic acid, ellagic acid, galloyl glycosides, mangiferin, and isomangiferin (Barreto et al., 2008; Manthey and Perkins-Veazie, 2009; Ribeiro et al., 2008). In general, the total phenolic compounds are reflected as galic acid equivalent (GAE). The concentration of phenolics in mango pulp ranges between 9 - 209 mg GAE·100 g^{-1} FW, depending on cultivar and ripening stage (Ma et al., 2011; Manthey and Perkins-Veazie, 2009; Ribeiro et al., 2007; Sulaiman and Ooi, 2012; Wu et al., 2004). During ripening, some cultivars do not change (Kim et al., 2010; Sulaiman and Ooi, 2012), while others increase or decrease in phenolic content (Kim et al., 2007; Maciel et al., 2011; Sulaiman and Ooi, 2012).

Ascorbic acid is an organic acid with antioxidant property which naturally occurs in fruit and vegetables. Higher ascorbic acid content was detected in mature green mango compared to that in ripe fruit. As much as 50% lost in ascorbic acid from the initial development to fully ripe mango fruit was reported by Gomez and Lalojo (2008). Sulaiman and Ooi (2012) reported that the highest ascorbic acid content was found in mature green mango fruit that is 256 μg AAE·g^{-1} sample and decreased to 170 μg AAE·g^{-1} in ripe fruit. Ascorbic acid contents in ripe 'Tommy Atkins' and 'Ubá' mango fruit were 10 and 78 mg·100 g^{-1}, respectively (Ribeiro et

al., 2007). Manthey and Perkins-Veazie (2009) reported similar ascorbic acid content in ripe 'Tommy Atkins' and 'Ataulfo' mangoes, that is 19.3 and 125.4 mg·100 g^{-1}, respectively. Other mango cultivars, such as 'Keitt', 'Kent', and 'Haden' (24.7, 25.6, and 31.0 mg·100 g^{-1}, respectively) contain lower ascorbic acid compared to 'Ataulfo' and 'Ubá' (Manthey and Perkins-Veazie, 2009). In addition, ascorbic acid contents of eight different mango cultivars grown in China at their mature stage range between 19.79 and 34.59 mg·100 g^{-1} (Ma et al., 2011). Cultivars or genetic factors seem to contribute to the variations in ascorbic acid content of mango fruit besides the maturity stage.

Polyphenols, ascorbic acid, and carotenoids are known as natural antioxidants found in fruit and vegetables. The variability in antioxidant capacity of fruit is due to the variability in composition of the antioxidant compounds in different species and cultivars of fruit (Award et al., 2001; Kondo et al., 2004a; Ma et al., 2011; Manthey and Perkins-Veazie, 2009) and the method used in assaying the antioxidant capacity (Gorinstein et al., 2010; Ma et al., 2011). Total antioxidant capacity of mango fruit evaluated by oxygen radical absorbance capacity (ORAC) method was 10.02 μmol TE·g^{-1} (Wu et al., 2004). Antioxidant activity measured by DPPH method in mature green mango cv. 'Chokanan' was 408.21 μg TE·g^{-1}, which was higher than that in the ripe fruit and other mango species and cultivars (Sulaiman and Ooi, 2012). Lost in DPPH reduction values was also reported during ripening of mango fruit, where some cultivars lost half of their initial antioxidant capacity (Manthey and Perkins-Veazie, 2009). Similarly, lower antioxidant capacity in ripe mango fruit compared to its mature green fruit was measured by Ferric Reducing Antioxidant Potential (FRAP) method (Sulaiman and Ooi, 2012). Compared to other cultivars, 'Ataulfo' is the richest cultivar concerning its phenolic compounds and ascorbic acid content (Manthey and Perkins-Veazie, 2009; Ribeiro et al., 2008). Mango cv. 'Tainong' showed the highest antioxidant capacity, about 2.1 – 6.2 times higher than other genotypes, regardless the assessment methods. Polyphenolic compounds are the major contributor to this antioxidant capacity of mango fruit (Ma et al., 2011).

Pre- and postharvest treatments can affect the antioxidant compounds and their activity. Antioxidant activity and phenolic compounds in all maturity stages of mango fruit derived from biodynamic farming were higher compared to mangoes from conventional farming (Maciel et al., 2011); storage temperature influenced the

ascorbic acid content of 'Alphonso' mango, i.e. fruit stored below room temperature had higher content than those at room temperature (Thomas, 1975); the use of electron-beam ionising radiation stress (1 – 3.1 kGy) delayed the reduction of the ascorbic acid and carotenoids concentration during storage of 'Tommy Atkin' (Reyes and Cisneros-Zevalos, 2007).

2.3. Controlled atmosphere

Controlled atmosphere (CA) storage involves reducing O_2 levels and increasing CO_2 levels in the storage atmosphere and maintaining their precise control. The atmosphere composition of normal air is 78% nitrogen, 21% O_2, and 0.03% CO_2 whereas in CA storage generally O_2 is reduced to below 8% and CO_2 is increased to above 1% (Brecht et al., 2003; Kader, 2005a; Mathooko, 1996b). Reducing O_2 and increasing CO_2 delays ripening process, reduces respiration and C_2H_4 production rates, hence reducing softening and decelerating numerous compositional changes. Most of the valuable effects of CA storage are due to low O_2 concentration (Thompson, 1998; Mahajan, et al., 2014). Other factors that are also controlled in CA storage are temperature, relative humidity, concentration of C_2H_4, and exposure time of the commodity (Saltveit, 2003). Adjustment in atmosphere composition and low temperature during storage has been proven to be more effective in maintaining mango fruit shelf life and quality (Singh and Singh, 2012; Singh and Zaharah, 2013).

Mango is one of the most desirable tropical fruit due to its special taste, aroma and nutritional value. However, as many other tropical fruit, mangoes are seasonal and delicate in nature having short shelf-life. Several efforts to prolong the postharvest life of mango have been explored. One of the most promising techniques is to control the atmosphere composition in storage. The first CA storage developed for mango was reported in 1937 when mango fruit were stored under reduced O_2 (9.2%) to extend their ripening period (Singh et al., 1937). In CA, the respiration, oxidative tissue damage or discoloration, rate of chlorophyll degradation and C_2H_4 production of fresh produce are reduced and the senescence process is delayed (Beaudry, 1999; Mathooko, 1996a, b; Reid, 1992). A stress response that contributes to physiological disorders of the commodity is the main problem arising when storage conditions fall beyond the optimum requirement (Kader, 2003a; Mahajan et al., 2014). Low O_2 and high CO_2 can induce anaerobic respiration and other

metabolic imbalances, leads to both external and internal damage (Bishop et al., 2003) such as off-flavour, reduction in aroma biosynthesis, and induction of tissue injury (Beaudry, 1999). The critical factor during storage is the actual concentration of O_2, CO_2, and C_2H_4 within the cells of the products (Kays and Paull, 2004).

Responses of fruit to CO_2 vary depending on the sensitivity of the tissue to the gas, exposure time, and temperature. Under elevated CO_2, the control of respiratory metabolism is determined by the enzyme-substrate availability (Watkins and Zhang, 1998). Pre-climacteric 'Kent' and 'Tommy Atkins' mango fruit after storage in more than 45% CO_2 atmosphere showed significant lower respiration rate compared to normal atmosphere storage (Bender and Brecht, 2000). Mature green 'Kensington Pride' mango ripened at 21°C for 7 days exhibited a low respiration rate after CA storage for 21 days (Lalel et al., 2001). Storage of mangoes for 3 weeks in 76 or 152 mmHg under low O_2 level at 13°C resulted in normal ripening process and higher percentage of consumable fruit (Spalding and Reeder, 1977). The rate of respiration of 'Delta R2E2' mango after CA storage was significantly lower than that stored under normal air in 13°C for 24 and 38 days. However, fruit stored in 6% CO_2 and 3% O_2 showed no significant difference of respiratory quotient and ripened normally with high TSS and total sugars compared to those in normal air (Lalel et al., 2005).

2.3.1. Effect of CA storage on carbohydrate and organic acid metabolism

There are three major interacting pathways involve in the respiratory oxidation of carbohydrate i.e. glycolysis, tricarboxylic acid (TCA) cycle, and electron transport system. The first pathway is for complex carbohydrates to be broken down into pyruvic acid, which is taken place in the cytoplasma and does not require O_2. The second pathway occurs in the mitochondria where the three-carbon compound from glycolysis is oxidized to CO_2. The third step involves in the transport of hydrogen atoms through a series of oxidation-reduction reactions to produce water. Another pathway that is known as an alternative means for the oxidation of sugars is the pentose phosphate pathway. Oxygen is essential for the last three pathways to proceed (Kays, 1991). Under anaerobic condition, the TCA cycle is inhibited and pyruvate is converted into lactate, acetaldehyde, and ethanol (Kays and Paull, 2004). The acetaldehyde and ethanol have shown their effect on retarding the senescence and inhibiting the C_2H_4 production in various fruit although

species and variety of fruit may react differently (Pesis, 2005). It was reported that carbohydrates in fruit are completely oxidised to CO_2 and decarboxylated end products of glycolysis, but were not accumulated during storage under very low O_2 (3 to 5 kPa) concentrations (Chervin et al., 1996).

The starch content in 'Haden' and 'Tommy Atkins' mangoes was completely reduced during 3 weeks of CA (2% – 5% O_2) storage at 12°C, but insignificant lower sugar level in CA-stored fruit compared to normal air-stored fruit after 5 days at 20°C was reported by Bender et al. (2000c). Under similar conditions, 'Alphonso' and 'Banganapalli' mangoes were stored for 4 weeks in CA (3 and 5% O_2) storage. The total soluble solids (TSS) and sugar contents of these two cultivars increased significantly, which demonstrated the initiation of ripening during CA storage (Rao and Rao, 2008). In contrast, 'Palmer' mangoes stored in CA under low O_2 concentration (1%, 5%, and 10%) contained lower TSS (Teixeira and Durigan, 2011).

Titratable acidity (TA) in 'Haden' and Tommy Atkins' mangoes increased two times in CA-stored fruit than normal air stored for 3 weeks at 12°C, whlist CA-stored fruit maintained higher TA level compared to normal air-stored fruit after 5 days of ripening at 20°C (Bender et al., 2000c). Low reduction rate of TA in mango fruit has been reported when fruit was stored in 3% O_2 and 0% or 10% CO_2 compared to normal air-stored fruit after 2 weeks at 10°C (Kim et al., 2007). Similar results have been reported in mango cv. 'Alphonso', 'Banganapalli', and 'Tommy Atkins' during CA storage at 13°C. Low uptake of organic acids as substrates for respiration during CA storage could be the reason of this phenomenon (Bender et al., 2000c; Rao and Rao, 2008). Coating is another postharvest practice in reducing the O_2 availability. Titratable acidity in coated mangoes cv. 'Tommy Atkins' was significantly higher compared to uncoated fruit after 45 days storage at 4°C due to the reduced respiration and low O_2 (Madeiros et al., 2012).

Overall, the phenomenon of carbohydrate hydrolysis and organic acids accumulation gave a picture of fruit ripening that proceeds regardless of the atmosphere conditions; however the time to reach the eating ripe stage is extensively prolonged under CA condition. An in-depth research on the effect of CA composition on sugars and acids contents in 'Kensington Pride' mangoes during storage followed by ripening is required.

2.3.2. Effect of CA storage on fruit texture

Tissue softening is a crucial phenomenon which occurs during storage and ripening of mango fruit as described in section 2.2.4. Research on 'Kensington Pride' mango fruit stored under reduced O_2 and increased CO_2 composition at 12 - 12.5°C suggested that changes in fruit firmness were affected by reduced O_2 rather than by increased CO_2. Oxygen concentration below 5% significantly retained fruit firmness (McLauchlan and Barker, 1992). 'Palmer' mango preserved their initial firmness for 28 days when stored under reduced O_2 level (1% - 10%) at 12.8°C. The low soluble pectin content in fruit was also recorded during CA storage under low oxygen level. 'Keitt' mango fruit stored for six days in 0.3% O_2 at 20°C prior to modified atmosphere storage showed a delay in fruit softening (Gonzalez-Aguilar et al., 1997). However, the firmness decreased significantly when fruit stored under 15% and 21% O_2 (Teixeira and Durigan, 2011). Fruit firmness reduced after CA storage (5 or 3% O_2 combined with 5% or 3% CO_2) in 'Alphonso' and 'Banganapali' mango but the CA stored fruit were firmer compared to fruit stored in normal atmosphere at 13°C (Rao and Rao, 2008). A reduction in fruit firmness was reported by Lalel et al. (2003a) when 'Kensington Pride' mango fruit were stored in 2% O_2 combined with 2, 6 or 9% CO_2 for 3 and 7 weeks, resulted in significant softer fruit than normal atmosphere-stored fruit at 13°C. The effect of CA storage on fruit firmness retention have been reported in other crops, such as apple (Ingham et al., 1998), kiwifruit (Botondi et al., 2011), cherimoya (Alique and Oliveira, 1994), peach (Bonghi et al., 1999). The retention of mango fruit firmness during CA storage has been suggested to be connected to the reduced activity of pectin and cell wall degrading enzyme (Rao and Rao, 2008). However, no research work has been reported on the effect of CA storage on the enzymes related to fruit softening in 'Kensington Pride' mangoes during storage and ripening, hence warranting to be investigation.

2.3.3. Effect of CA storage on colour changes

Colour of fruit is the mirror of pigments present in fruit. In many fruit, colour change is the indicator of ripening. Colour of 'Palmer' mangoes was not affected by CA with low O_2 level since they showed no significant difference to those stored in normal atmosphere at 12.8°C (Teixeira and Durigan, 2011). Chlorophyll degradation of 'Kensington Pride' mango was slowed down by decreasing O_2 concentration from

10% to 2% and increasing CO_2 from 0 to 4%. It was also showed that further increase in CO_2 concentration did not affect colour (McLauchlan and Barker, 1992). During 35 days in CA storage 'Kensington Pride' mango fruit showed significant increase in β-carotene content under 9% CO_2 and 2% O_2 (Lalel et al., 2001) and increase concomitantly with increasing CO_2 concentration in storage (Lalel et al., 2003a). However, it has been reported that CO_2 concentration beyond 25% impaired the development of mango skin colour (Bender et al., 1994). A delay in chlorophyll degradation of 'Delta R2E2' mango has been reported by Lalel et al. (2005) during CA storage. 'Alphonso' and 'Banganapalli' mangoes stored under CA retained its skin chlorophylls due to the reduced activity of chlorophyll degrading enzyme; however the carotenoids content in mango pulp was increased (Rao and Rao, 2008). In contrast, Kim et al. (2007) reported that CA composition of 3% O_2 combined with 0% or 10% CO_2 delayed the colour development in mango pulp. Lower concentrations of carotenoids were found in 'Alphonso' mango during storage in CA composed of 5% O_2 and 5% CO_2 for 45 days at 8°C, compared to those stored in regular air (Niranjana et al., 2009).

During ripening, 'Alphonso' and 'Banganapalli' fruit retreated from 5% O_2 experienced natural ripening process within one week under ambient condition while those from 3% O_2 resulted in significantly lower carotenoids formation (Rao and Rao, 2008). The colour development of 'Palmer' mango fruit retrieved from CA storage with 1%, 5%, and 10% O_2 appeared within 8 days at 25.2°C without any CA storage defects (Teixeira and Durigan, 2011). The effect of CA storage on degradation of chlorophylls and synthesis of carotenoids in 'Kensington Pride' mango fruit during storage and ripening is important to be investigated.

2.3.4. Effect of CA storage on nutritional quality

Mango fruit is a good source of dietary antioxidants such as ascorbic acid, carotenoids, and phenolic compounds (Ma et al., 2011). The reduction of total polyphenolic compounds in mature green mango cv. 'Tommy Atkins' was minimised by CA storage (3% O_2 + 97% N_2 and 3% O_2 + 10% CO_2 +87% N_2) for 14 days at 10°C, but naturally decreased during ripening at normal air (Kim et al., 2007). Higher concentration of phenolics was also retained in 'Alphonso' mango

fruit stored in CA (5% O_2 + 5% CO_2) for 45 days at 8°C compared to those fruit stored in ambient conditions (Niranjana et al., 2009).

Ascorbic acid contributes to the antioxidant activity in mango fruit (Manthey and Perkins-Veazie, 2009). Rao and Rao (2008) reported that ascorbic acid content in hot water-treated 'Alphonso' and 'Banganapali' reduced significantly after CA storage compared to those stored in normal atmosphere for 4 weeks at 13°C, and further decreased was discovered during ripening at 25 – 32 °C; while the degree of reduction was significantly different between those mango cultivars. In other crops, CA has shown to affect ascorbic acid content. Kiwifruit (*Actinidia deliciosa* cv. 'Hayward') at different maturities was stored in CA (2% O_2 + 5% CO_2) for 5 months at 0°C. The maturity stages and atmosphere composition during storage have significant effect on the reduction of ascorbic acid content. Ascorbic acid level was reduced more extensively in early-harvested fruit than in late-harvested fruit during CA storage whereas those stored in normal atmosphere condition can preserve their ascorbic acid (Oz, 2010). 'Conference' pears, subjected to CA (2% O_2 + 5% CO_2) stored for 6 months, demonstrated a fast decrease in total ascorbic acid content compared to those stored for the same period of time but under atmosphere composed of 2% O_2 + 0.7% CO_2 and normal air (Larrigaudiére et al., 2001).

2.4. Roles of methyl jasmonate (MJ) on textural and biochemical changes

Ripening of fruit is the result of multiple hormone interaction, either synergistic or antagonistic (Pech, 2012; Khan et al., 2015). Jasmonates, including jasmonic acid (JA) and methyl jasmonate (MJ), are known to promote embryo development, fruit ripening, senescence, and accumulation of storage protein (He et al., 2002; Mukkun and Singh, 2009; Ziosi et al., 2008), in addition to its capability in protecting against pest and pathogens (Cao et al., 2008; Peña-Cortés et al., 2005). They are known as the signalling molecules that respond to environmental stresses, biotic and abiotic stresses (Khan et al., 2015; León and Sánches-Serrano, 1999; Wasternack, 2007). As a natural plant growth regulator, MJ is an important biological substance in regulating biosynthesis of secondary metabolites (Creelman and Mullet, 1997), which is essential in human health.

2.4.1. Endogenous methyl jasmonate

Jasmonanic acid and its volatile ester MJ are synthesized naturally in both climacteric and non-climacteric fruit, promoting C_2H_4 production and aroma in climacteric fruit (Peña-Cortés et al., 2005). The concentration of this compound in plants varies with tissue or cell type, level of development, and in response to environmental stress (Creelman and Mullet, 1997). Initially, jasmonates, the collective name for JA and MJ, are synthesized through octadecanoid pathway from α-linoleic acid converted to 12-oxo-phytodienoic acid (OPDA) by multiple enzymes such as lipoxygenase (EC 1.13.11), allene oxide synthase (EC 4.2.1.92) and allene oxide cyclase (EC 5.3.99.6) in the chloroplast. The reduction of OPDA to jasmonates is triggered by cytoplasmic 12-oxo-phytodienoic acid reductase and 3 cycles of β-oxidation in the final step of the biosynthetic reactions which take place in peroxisomes (Creelman and Mullet, 1997; León and Sánches-Serrano, 1999; Wasternack, 2007; Ziosi et al., 2008). JA is then catabolised by JA carboxyl methyltransferase to form MJ, the volatile substance of JA (Cheong and Choi, 2003). Besides fruit ripening, jasmonates can also modulate fruit carotenoid composition as reported by Creelman and Mullet (1995; 1997).

Kondo et al. (2004b) determined the internal JA concentration in 'Nam Dok Mai' and 'Nam Klangwan' mango fruit started from the developmental stage to ripening. The JA content was high at the early developmental stage, decrease with days after full bloom, and rise again during fruit ripening. The increasing amount of JA during storage and ripening is reported to be associated with the moisture loss in mango fruit within this period. Endogenous JA in 'La France' pears was higher than C_2H_4 concentration at the beginning of ripening. However, the ethylene content of pears rose on the third day while JA content reduced within 9 days in ripening period before increased to the same level as ethylene on day 18 (Kondo et al., 2007). Endogenous MJ, as *trans*-MJ, in strawberry was determined at different level of fruit development and ripening. The highest MJ concentration was found in white fruit, followed by half ripe then fully ripe fruit. However, the MJ concentration decreased in all fruit within 6 days during postharvest period, and the trend was more obvious in white fruit (80%) compared to half ripe (37%) and fully ripe (7.6%) fruit (Mukkun and Singh, 2009). The fruit growth stages have been proposed as an important factor

that influences the different response to JA (Kondo et al., 2007; Mukkun and Singh, 2009).

2.4.2. Exogenous application of methyl jasmonates

Exogenous application of jasmonate stimulates transient and large changes of its endogenous concentration in plant cells. It activates numerous gene expression, influences plant growth and responses to stresses. Synthesis and accumulation of jasmonate inside the chloroplast prevents the level of jasmonate in other compartment to rise. Under this manner, the plants are able to control gene expression. However, when JA accumulation in plant initiated through over-expression of allene oxide synthase, the gene expression was hampered (Creelman and Mullet, 1997).

2.4.2.1. Ethylene production and respiration

The postharvest MJ treatment (vapour concentration at 10^{-3} M and 10^{-5} M) of 'Kensington Pride' mangoes showed a climacteric increase of respiration and C_2H_4 production during ripening as compared to the control fruit (Lalel et al., 2003g). MJ-treated and untreated mango fruit cv. 'Nam Dok Mai No. 4' was able to ripe normally after 21 days in cold storage but MJ-treated fruit exhibited 10 days delayed fruit ripening (Junmatong et al., 2012). In other crops, exogenous application of MJ stimulates C_2H_4 production regardless of the developmental stages of tomato fruit (Saniewski et al., 1987) by activating ethylene-forming enzyme (Czapski and Saniewski, 1992). The application of MJ significantly increased the ethelyne production of strawberry in all maturity levels, although it was lower in fully ripe fruit (Mukkun and Singh, 2009). The increase in C_2H_4 production could be due to the enhanced activation of ACC synthase and ACC oxidase (Fan et al., 1998b; Kondo et al., 2007; Mukkun and Singh, 2009) which are known as the ethylene-forming enzyme. In guava fruit, application of MJ increased PAL activity and the fruit was able to ripe normally after 5°C storage, indicating a positive response to overcome stress (Gonzalez-Aguilar et al., 2004). However, postharvest application of MJ (10 $\mu mol \cdot l^{-1}$ in sealed chambers for 24 hours at 20°C) on loquat fruit inhibits respiration and C_2H_4 production, decreases the activity of phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) during 35 days storage (Cao et al., 2009a).

2.4.2.2. Fruit softening

Jasmonates have been known to influence the process of fruit softening. Fruit softening was retarded in mango cv. 'Kent' treated with 10^{-5} M MJ during 14 days storage at 10°C followed by 7 days ripening at 20°C compared to untreated fruit (Gonzalez-Aguilar et al., 2001). Mango fruit cv. 'Nam Dok Mai No. 4' dipped into MJ (0.1 and 1 mM) and stored for 42 days at 5°C were firmer and their ripening ambient temperature was prolonged to 35 days, whilst untreated fruit showed chilling injury (CI) symptom after 21 days and deteriorated after 28 days (Junmatong et al., 2012). Peach fruit treated with 0.1 $mmol \cdot l^{-1}$ and stored at 5°C for 21 days maintained firmness for longer than those stored at 10°C although the firmness decreased within 3 days in shelf life (Meng et al., 2009).

In conjunction to fruit firmness, the mode of textural change during ripening is well known, as described in section 2.2.4. However, little is known on the activity of softening enzymes affected by exogenous application of MJ.

2.4.2.3. Fruit quality

Tasneem et al. (2004) has reported that MJ treatment of mango fruit reduced weight loss during storage at 7 and 10°C. Ripe mango fruit developed brighter skin colour, and higher TSS value than untreated fruit within 4 days of ripening at 20°C. High accumulation of sugar in treated-ripe fruit after storage at 5°C indicated that jasmonate treatment was able to maintain the carbohydrate biosynthesis under stress condition (Kondo et al., 2005a). Mango fruit cv. 'Kent' treated with 10^{-5} M MJ vapour for 20 hours at 20°C demonstrated skin degreening, and reduction in weight losses during 14 days storage at 10°C followed by 7 days ripening at 20°C (Gonzalez-Aguilar et al., 2001). MJ treatment was reported to increase the yellow colour on the fruit skin of mango cv. 'Kensington Pride' and 'Kent' (Khan et al., 2015). Lalel et al. (2003g) reported the development of yellow skin colour of 'Kensington Pride' mangoes vaporized with 10^{-3} M MJ. Contents of fatty acids, total aroma volatiles, monoterpene, sesquiterpene, norisoprenoid, alcohols, and esters in fruit pulp, while the accumulation of *n*-tetradecane was reduced. The role of exogenous MJ at the initial step of mango fruit ripening has been suggested (Singh et al., 2013).

The application of MJ (10 μmol·l^{-1}) to loquat fruit resulted in higher sugars, organic acids, total phenolic and flavonoids than untreated fruit during 35 days of storage at 1°C. The antioxidant capacity of fruit measured by 2,2-diphynyl-1-picrylhydrazyl (DPPH) radical scavenging activity was in significant positive linear relationship with the total phenolic content (Cao et al., 2009b). In guava fruit, application of MJ has a positive correlation with increasing ascorbic acid and accumulates sugars after 5°C storage (Gonzalez-Aguilar et al., 2004). Jasmonates treatment promoted degreening of fruit skin with minimal loss in quality in apple fruit. TA in fruit treated with JA was lower than control fruit, although TSS and fruit firmness were not affected by Jasmonates treatment. The effect of JA on colour changes in apple fruit depended on the developmental stages of fruit and dose (Fan et al., 1998a). Higher activity of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT), and ascorbate peroxidase (APX) and lower lipoxygenase (LOX) activity were also measured in MJ-treated loquat fruit than in untreated fruit (Cao et al., 2009a). Increasing antioxidant enzymes activity, antioxidant capacity, and free radical scavenging capacities in fruit tissues after MJ treatment is related to the capability of this compound in preventing the tissues from free radical injury (Wang, 2006). High activity of peroxidase and low phenolic compound were reported in peach fruit treated with MJ and stored at 5°C for 3 weeks before transfer to 20°C. The treated fruit also retained higher TSS/TA ratio which was due to lower TSS reduction compared to control fruit (Meng et al., 2009). Exogenous MJ treatment improved the overall quality and storage life of berry fruit such as raspberries, strawberries, and blackberries (Ayala-Zavala et al., 2005; Chanjirakul et al., 2006, 2007; Wang et al., 2008b; Wang and Zheng, 2005).

The application of MJ in climacteric and non climacteric fruit has been shown to influence the process of fruit ripening and eating quality. However, the role of MJ in combination with CA storage has not been reported so far on any fruit commodity. It is therefore important to study the interaction of JA with storage atmosphere to deteremine if synergistic effects could lead to positive outcomes on storage stability and fruit quality in mango.

2.5. Roles of polyamines on textural and biochemical changes

Polyamines (PAs), the aliphatic amines, are endogenously synthesized in all living cells through a highly regulated pathway (Bouchereau et al., 1999; Kakkar and

Rai, 1993). These are known to modulate a wide range of biological processes, including plant growth, flowering, fruit ripening, stress responses, senescence, secondary metabolite production, and enzyme activities (Bouchereau et al., 1999; Fariduddin et al., 2013; Kakkar and Rai, 1993; Kussano et al., 2008; Malik et al., 2003; Tiburcio et al., 2003). The presence and concentration of these compounds depends on the biosynthesis, conjugation, degradation, conversion to other metabolites, plant species, organs, tissues, and also developmental stages (Kakkar and Rai, 1993; Tiburcio et al., 2003).

2.5.1. Endogenous polyamines

PAs are present in all parts of plant and distributed in all compartments of plant cell (Kussano et al., 2008). The presence of PAs in plant tissue is much higher than other endogenous phytohormones which range between 10^{-9} – 10^{-5} M compared to 10^{-13} – 10^{-7} M, respectively (Fariduddin et al., 2013). It was reported that PAs biosynthesis are significantly increased under increasing stress condition (Franchin et al., 2007). The endogenous level of PAs in fruit could also be increased through exogenous PAs treatment (Torrigiani et al., 2008; Xu et al., 2001).

Endogenous PAs in fruit are putrescine (Put: butane-1,4-diamine), spermidine (Spd: N-(3-amino propyl) butane-1,4-diamine), and spermine (Spm: NN'-bis-(3-aminopropyl) butane-1,4-diamine) (Malik and Singh, 2004; Malmberg et al., 1998; Santiago-Silva et al., 2011). PAs are synthesized through decarboxylation of amino acid such as arginine (ARG) and ornithine (ORN) for the carbon skeleton while methionine (MET) synthesized *S*-Adenosynmethionine (SAM) to ultimately form Spd and Spm (Figure 2.2.). Two pathways are involved in Put biosynthesis: decarboxylation of ARG by arginine decarboxylase (ADC) and decarboxylation of ORN by ornithine decarboxylase (ODC) (Bagni, 1986; Hamdani et al., 2011; Kaur-Sawhney et al., 2003). Decarboxylated SAM (dcSAM) supplies aminopropyl group for synthesis of Spd and then Spm which are catalysed by Spd and Spm synthases, respectively (Hamdani et al., 2011; Kaur-Sawhney et al., 2003). PAs produced directly through ODC take part in regulation of cell division, while those synthesised indirectly through ADC involve in plant vegetative development (Cohen et al., 1983).

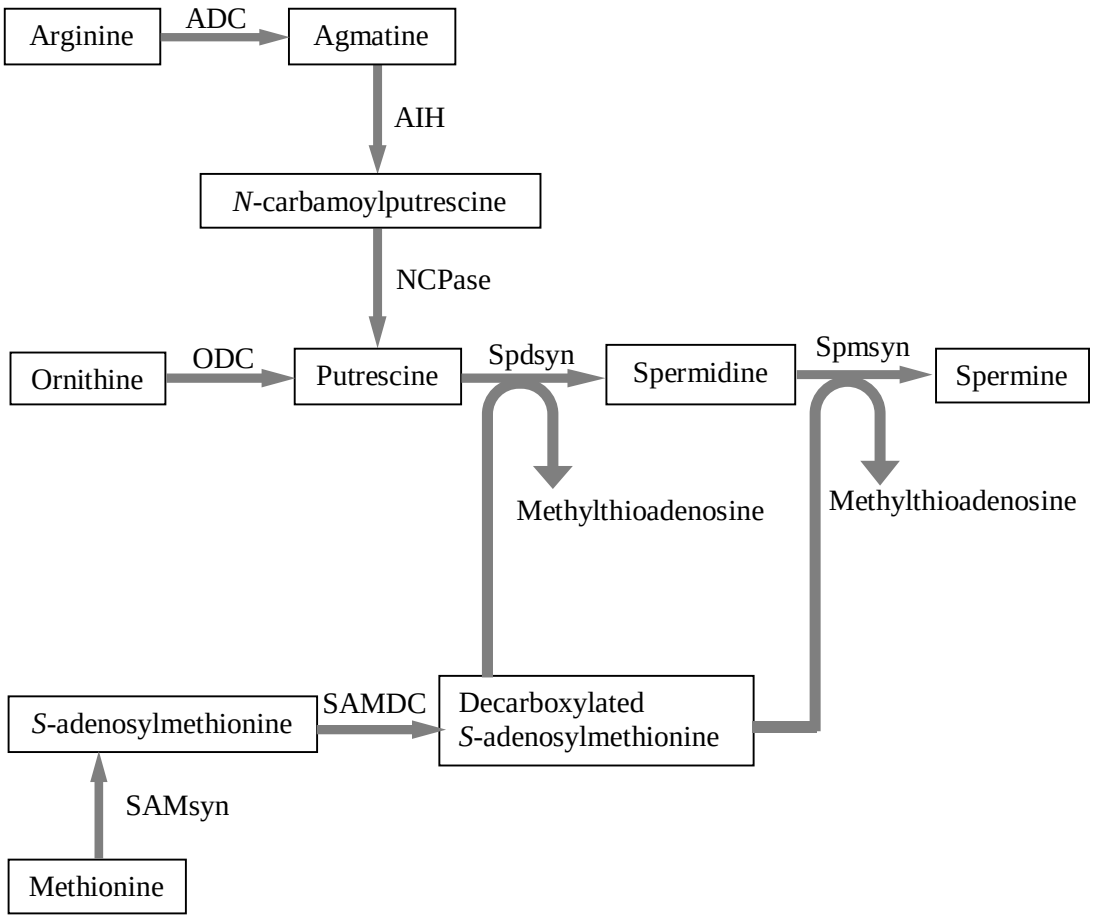

Figure 2.2. Biosynthesis of polyamines (PAs) in plant: ADC, arginine decarboxylase; AIH, agmatine iminohydrolase; NCPase, *N*-Carbamoylputrescine amidohydrolase; ODC, ornithine decarboxylase; SAMDC, *S*-adenosylmethionine decarboxylase; SAMsyn, *S*-Adenosulmethionine synthase; Spdsyn, spermidine synthase; Spmsyn, spermine synthase (redrawn from Tiburcio et al., 2003).

It is well understood that PAs share a common precursor with C_2H_4, SAM, for their biosynthesis. However, their roles in fruit ripening and senescence are somehow antagonistic (Hu et al., 2006; Pandey et al., 2000). Increase in C_2H_4 concentration, either through exogenous application or biosynthesis, has been reported to retard PAs synthesis (Apelbaum et al., 1985; Bregoli et al., 2002; Icekson et al., 1986) whereas PAs obstruct C_2H_4 production (Apelbaum et al., 1985; Paksasorn et al., 1995; Perez-Vicente et al., 2002; Serrano et al., 2003). This phenomenon indicates a possibility of competition in biosynthesis as reported in pears and peaches (Bregoli et al., 2002; Ohkawa et al., 1998).

However, different conclusion was acquired when inhibitors of PAs and C_2H_4 were used in studying their effects during ripening of banana fruit, where the

synthesis of C_2H_4 in wounded tissues involves in the induction of ADC and accumulation of Put; therefore C_2H_4 and PAs may not compete for SAM in banana fruit (Yosa et al., 1996). Similarly, a study on tomato fruit revealed that Put content increased together with the restoration of gene expression of C_2H_4 receptor, hence it is not a ripening inhibitor compound, but it may extend the fully-ripe stage hence delay the senescence process (Tassoni et al., 2006). PAs were more likely to involve in regulating the cell homeostatis rather than to compete with C_2H_4 for SAM in their biosynthesis (Dias et al., 2010).

Free PAs was found in mango cv. 'Tommy', passion fruit, and pineapple with high average level of Spd followed by Spm than Put. The average concentration of Put was the highest among PAs in papaya, followed by Spd and Spm. In red guava, Spm was the major PAs followed by Spd and than Put as the minor one (Santiago-Silva et al., 2011). In avocado, high PAs levels was found until maturity stage (Apelbaum, 1986), while in pears and mandarins, the highest amount of PAs was at the initiation of cell division (Nathan et al., 1984; Toumadje and Richardson, 1988). Increasing levels of PAs during fruit ripening have been reported by different researchers on mandarins, cherimoya, and rambutan (Escribano and Merodio, 1994; Kondo et al., 2001; Martines-Madrid et al., 1999; Nathan et al., 1984). The concentration of PAs is related to fruit development (Kakkar and Rai, 1993), ripening and maturity stage (Barrachina et al., 2000), and cultivars (Zuzunaga et al., 2001). Moreover, the type of PAs varies with species, growth habits and phenological stage (Biasi et al., 1991; Santiago-Silva et al., 2011).

2.5.2. Exogenous application of polyamines

The exogenous application of PAs could be delivered in different techniques and at different stages of fruit growth. Pre-harvest treatments of PAs on flower and on fruit were usually done by spraying, whereas postharvest treatments were employed by pressure infiltration or by immersing fruit to the PAs solution. Moreover, species and cultivars may respond differently to the type and concentration of PAs. In general, exogenous application of PAs results in improved fruit quality (Khan and Singh, 2007a; Kramer et al., 1991; Martínez-Romero et al., 2002; Mirdehghan et al., 2007; Srivastava and Ahmad, 2014; Winer and Apelbaum, 1986); hence prolonging the shelf-life of fruit. The effects of PAs delivered through different methods and fruit growth stage on various fruit will be reviewed.

2.5.2.1. Ethylene production and respiration

Pre- and post-harvest PAs treatments in various fruit have reduced C_2H_4 production and respiration rate (Khan and Singh, 2007b; Kramer et al., 1991; Law et al., 1991; Martínez-Romero et al., 2002; Mirdehghan et al., 2007; Perez-Vicente et al., 2001; Perez et al., 1993; Winer and Apelbaum, 1986), hence delaying senescence and prolonging the shelf-life of fruit.

Polyamines treatment on mango fruit cv. 'Kensington Pride' (Malik et al., 2003) and cv. 'Samar Bahisht Chaunsa' (Razzaq et al., 2014) inhibited the synthesis of C_2H_4 whereas concentration of PAs increased concomitantly with climacteric rise of C_2H_4 during mango fruit ripening (Malik and Singh, 2004). Applied PAs prior to storage also reduced C_2H_4 production and respiration rate in avocado (Winer and Apelbaum, 1986), plum (Ren et al., 1995; Perez-Vicente et al., 2001; Perez-Vicente et al., 2002), peach (Martínez-Romero et al., 2000), apricot (Martínez-Romero et al., 2001; Saba et al., 2012), and pomegranate (Mirdehghan et al., 2007).

Delayed C_2H_4 emission was measured in peach (Bregoli et al., 2002) and plum (Khan and Singh, 2007b) treated with PAs prior to harvest. The reduction in C_2H_4 synthesis was progressively decreased as the concentration of Put increased (Khan and Singh, 2007b) indicated an opposite relationship between PAs concentration and C_2H_4 production (Singh et al., 2013). Pre- and or post-harvest PAs treatments of apricot (Martínez-Romero et al., 2002; Torrigiani et al., 2004), tomato (Arava et al., 2000), peaches and nectarines (Bregoli et al., 2006) have been reported to inhibit the C_2H_4 production and delayed the ripening process. Different effects of PAs treatment on C_2H_4 synthesis and fruit quality when applied at different levels of fruit development indicated that the effective actions of PAs depend on ripening stage and cultivar (Bregoli et al., 2006).

2.5.2.2. Fruit softening

Manipulation of PAs concentration in fruit inhibited flesh softening process and cell wall degrading enzymes activities (Torrigiani et al., 2008). Exogenous applications of PAs have reduced softening and decay in various fruit, hence prolonged the shelf-life (Kramer et al., 1991). Mango fruit exposed to PAs treatment delayed softening during storage and maintained the firmness of ripe fruit with insignificant reduction in C_2H_4 evolution during ripening (Singh et al., 2013).

Together with maintaining fruit texture, reducing cell wall enzymes activities in mango fruit cv. 'Samar Bahisht Chaunsa' has also been reported (Razzaq et al., 2014). PAs delayed fruit softening in apricot, peach, papaya, and plum (Saba et al., 2012; Bregoli et al., 2002; Purwoko et al., 1998; Perez-Vicente et al., 2002; Khan and Singh, 2007b)

2.5.2.3. Fruit quality

Increased concentration of PAs in fruit may improve the nutritional quality including antioxidant capacity under specific circumstances (Torrigiani et al., 2008). Exogenous application of PAs on mango fruit delayed colour development during storage with insignificant reduction in C_2H_4 production during ripening (Singh et al., 2013) may have important value on mango fruit shelf life. Malik and Singh (2006) reported that the effects of exogenous application of PAs were influenced by the type and concentration of PAs, and the plant phenological stage. Pre-harvest application of Put at the final fruit set stage of 'Kensington Pride' improved ripe fruit quality by increasing pulp total carotenoids, sugars and TSS concentrations, while reducing acids content. Spd and Put treatment significantly reduced the ascorbic acid content in mango fruit by 24.2 and 20.6%, respectively. However, the ascorbic acid content was increased in ripe fruit treated with higher dose of Spm. PAs treatment at pre-harvest also delayed skin colour development compared to untreated fruit (Malik and Singh, 2006). The quality of Put-treated mango fruit cv. 'Samar Bahisht Chaunsa' was maintained better than untreated fruit during cold storage and ripening (Razzaq et. al., 2014). Pressure infiltration of Spm and Spd on 'Arumanis' mango fruit before ripening resulted in significantly better ripe fruit quality than those with Put (Purwoko et al., 1998).

Exogenous application of PAs on papaya fruit retarded weight loss and maintained fruit colour during ripening with lower TSS compared to untreated fruit (Purwoko et al., 1998). Spermine-treated tomato fruit preserved reducing sugars, high TSS value and acidity during storage (Arava et al., 2000). Pressure and immersion techniques were used during the application of Put or Spd (1 mM) on pomegranate before storage at 2°C. Both Put and Spm treatments equally improved fruit quality, including the TSS/acid ratio, and higher L^* value (Mirdehghan et al., 2007). The colour of apricot fruit infiltrated with Put prior to storage was delayed hence improve the storability of fruit (Martínez-Romero et al., 2001). Similarly, the

pre- and postharvest Put treatments of apricot have been reported to delay colour development (Martínez-Romero et al., 2002; Torrigiani et al., 2004).

Fresh produce quality management is one of the most essential factors for improving production and storage life of fruit and vegetables while any new technology is being exercised. It is important to maintain the fresh appearance of fruit and vegetables in order to meet consumer's demand. However, the new perspective of fresh quality has included undercover quality factors such as nutritional and functional properties (Saltveit, 1999). Application of exogenous PAs has revealed to delay fruit ripening, improve fruit composition and extend shelf life. They are also known to response to abiotic stresses such as environmental extremes (Bouchereau et al., 1999; Fariduddin et al., 2013; Groppa and Benavides, 2008; Pang et al., 2007; Perez-Vicente et al., 2002). However, to the best of our knowledge, no data has been reported regarding the effects of PAs in encountering O_2 and/or CO_2 stress under controlled atmosphere storage, and is yet to be evaluated in 'Kensington Pride' mango fruit.

2.6. Effects of 1-methylcyclopropene (1-MCP) on textural and biochemical changes

Ethylene is a plant hormone which controls a broad range of physiological process in plants including fruit ripening and senescence (Lelievre et al., 1997). Senescence and over-ripening that accelerates quantity and quality losses during storage of fresh produces are problems that hastened by C_2H_4 actions which have been well-studied. Thus, maintaining C_2H_4 production in postharvest products is an important issue for farmers and traders.

A novel technology in controlling C_2H_4 metabolism in plant produce is the application of 1- methylcyclopropene (1-MCP). 1-MCP is a cycloalkene (C_4H_6), a volatile gas at standard temperature and pressure. This odourless, non-toxic compound is a ripening inhibitor that obstructs the C_2H_4 perception sites in cell wall membrane of harvested plant or plant parts (Sisler and Serek, 1997; Yang and Hoffman, 1984), hence inhibiting C_2H_4 production (Watkins, 2008; Watkins and Miller, 2005). This gaseous compound delays softening and improves quality of several climacteric fruit during ripening (Blankenship and Dole, 2003). Despite of their similarity in the molecular structure, 1-MCP binds strongly and irreversibly to

the receptor whereas C_2H_4 diffuses easily and leaves 'ethylene-induced' symptoms. However, the ethylene of some products can be restored when a new receptor is formed (Sisler and Serek, 1997).

Several factors contribute to the effectiveness of 1-MCP treatment. These factors include both external and internal factors, such as the concentration and the exposure time, temperature, air composition in storage, genotype and level of maturity (Mahajan et al., 2014; Sisler and Serek, 1997; Watkins, 2008). High concentration of 1-MCP is required when applied under low temperature (Sisler and Serek, 1997, 2003).

2.6.1. Ethylene production and respiration

1-MCP has been an essential tool in controlling C_2H_4 in postharvest produces, including mango fruit (Blankenship and Dole, 2003; Mahajan et al., 2014; Martínez-Romero et al., 2007; Watkins, 2008; Watkins et al., 2000). The application of 1-MCP on mango fruit has been reported to inhibit C_2H_4 production and respiration, hence retaining firmness and delaying ripening of mango fruit (Hojo et al., 2006; Jiang and Joyce, 2000; Lalel et al., 2003f; Pandey and Singh, 2007; Santos et al., 2004; Singh and Dwivedi, 2008; Singh et al., 2011; Wang et al., 2009; Watkins, 2008).

Postharvest treatment of mangoes cv. 'Rosa', 'Jasmine' and 'Espada' with 1-MCP (100 $\mu l \cdot l^{-1}$) for 24 hours delayed C_2H_4 production (Silva et al., 2004). 'Kensington Pride' Mango fruit treated with 25 $\mu l \cdot l^{-1}$ 1-MCP for 6 or 14 hours reduced its C_2H_4 production and delayed in ripening by 5.1 days (Hofman et al., 2001; Lalel et al., 2003f). The treatment of 'Nam Dok Mai' mangoes with 0.5 $\mu l \cdot l^{-1}$ and 1.0 $\mu l \cdot l^{-1}$ 1- MCP for 24 hours prolonged fruit shelf life up to 15 days at 20°C (Penchaiya et al., 2006). Shelf life of mango cv. Guifei' was extended from 8 to 12 days when treated with 1-MCP at 5.0 $\mu l \cdot l^{-1}$ for 6 hours (Wang et al., 2006). The postharvest life of 'Tommy Atkins' mangoes, treated with 1-MCP (0.6 $\mu l \cdot l^{-1}$) for 12 hours at 25°C, was prolonged up to 21 days storage at 10 and 25°C (Bomfim et al., 2011). 1-MCP treatment at 0.3 $\mu l \cdot l^{-1}$ and 0.75 $\mu l \cdot l^{-1}$ failed to control respiration and C_2H_4 production in 'Kent' mangoes. The concentration, exposure period, and the temperature showed no effects on respiration and C_2H_4 emission in 'Kent' mangoes (Islas-Osuna et al., 2010; Osuna-García et al., 2009). Fruit ripening, C_2H_4

production and the expression of ACC synthase and several genes of C_2H_4 receptor in tomato fruit were delayed when treated with 1-MCP. However, the expression of C_2H_4 receptor genes restored as the fruit ripening was restarted (Tassoni et al., 2006), indicating a reinstatement of C_2H_4 production.

2.6.2. Fruit softening

Softening is a result of metabolic processes during fruit ripening. In climacteric fruit, softening is related to the degradation of cell wall matrices, specifically to the solubilisation of pectin compounds (Lashbrook, 2005; Lohani et al., 2004). Various factors are known to influence the effectiveness of exogenous application of 1-MCP on mango fruit firmness and quality. Those factors include concentrations of 1-MCP, cultivars, length and condition of storage, number of application and maturity levels (Alves et al., 2004; Cocozza et al., 2004a; Hojo et al., 2006; Lalel et al., 2003f; Lima et al., 2006; Mahajan et al., 2014; Santos et al., 2004; Silva et al., 2004; Singh and Neelam, 2008; Wang, 2006).

The fruit softening of 'Kent' mangoes exposed to 1-MCP at 0.3 $\mu l \cdot l^{-1}$ for 20 hours at 13°C was delayed (Osuna-García et al., 2009). However, higher concentration of 1-MCP (0.75 $\mu l \cdot l^{-1}$), shorter exposure time (12 hours) at higher temperature (20°C) failed to delay 'Kent' mango fruit softening during 14 days in storage (Islas-Osuna et al., 2010). Hot water treatment combined with 1-MCP application resulted in inhibition of softening, hence prolonged storage life of 'Keitt' mango fruit (Ngamchuachit et al., 2014). Hot-water treated 'Kent' mango fruit were exposed to 1-MCP (500 $nl \cdot l^{-1}$) for 12 hours at 20°C prior to 18 days CA storage at 10°C. On the fifth day of ripening, higher reduction in firmness was measured in fruit stored under 5% O_2 + 5% CO_2 than those under 3% O_2 + 8% CO_2. It was concluded that 1-MCP treatment worked synergistically with CA storage of 3% O_2 + 8% CO_2 to reduce softening of hot-water treated 'Kent' mango fruit (Sivakumar et al., 2012). Exogenous application of 900 $nl \cdot l^{-1}$ 1-MCP and stored at 11°C slowed down the softening of 'Tommy Atkins' mango fruit. Similar result was obtained when the same cultivar was treated at higher level of 1-MCP at higher temperature (1,200 $nl \cdot l^{-1}$ at 25°C) (Lima et al., 2006). Fruit firmness of 'Tommy Atkins' mangoes continuously decreased during storage, however fruit treated with 0.6 $nl \cdot l^{-1}$ 1-MCP showed lower reduction in firmness compared to those untreated and treated

with lower dose of 1-MCP (Bomfim et al., 2011). 'Tommy Atkins' mango fruit treated with 1-MCP at 100 $nl \cdot l^{-1}$ and packed in modified atmosphere resulted in higher fruit firmness losses compared to 1-MCP treatment alone (Cocozza et al., 2004a; Cocozza et al., 2004b).

In other cultivar, 1-MCP-treated 'Dashehari' mangoes were able to maintain its firmness better than the untreated fruit (Singh et al., 2007). Fruit softening of 'Nam Dokmai' mango was reduced by 1-MCP treatment. The reduction in fruit firmness was accompanied by decline in protopectin and increase in soluble pectin content (Penchaiya et al., 2006). 'Tainong' mangoes exposed to 1-MCP at 1 $\mu l \cdot l^{-1}$ for 24 hours and stored for 16 days at 20°C have better firmness than fruit treated with C_2H_4 (Wang et al., 2009), figuring the ability of 1-MCP in blocking the C_2H_4 receptor. The postharvest application of 1-MCP (100 $\mu l \cdot l^{-1}$) to mango for 12 hours alleviated the fruit softening process has also been reported by Jiang and Joyce (2000). 'Ataulfo' mango fruit subjected to 1-MCP at 400 $nl \cdot l^{-1}$ exhibited a reduction in weight loss and fruit softening within 14 days storage at 20°C (Rangel et al., 2009). Postharvest application of 1-MCP has maintained the concentration of sucrose and starch in 'Jinhwang" mango fruit (Wongmetha and Ke, 2012).

Softening-related enzymes activity in mango fruit treated with 1-MCP were inhibited in 'Chausa', 'Langra', and 'Dashehari' mango cultivars. The treatment of mangoes with 1-MCP at 1.0 $mg \cdot l^{-1}$ delayed accumulation of SSC in fruit which was due to the inhibition of PE and PG activity, and the level of inhibition was different among the three cultivars. Higher inhibition of PG activity was measured in 'Chausa' compared to 'Langra' and 'Dashehari' mangoes, whilst higher inhibition of PE activity was found in 'Dashehari' than other cultivars (Singh and Neelam, 2008). Similar report on the inhibition of softening enzymes (PE, PG, and α-D-galactosidase) in 'Palmer' mango in which the concentration of total soluble pectin and the degree of pectic substances solubilisation were also reduced by the application of 150 $nl \cdot l^{-1}$ MCP for 12 hours at ambient temperature until 18 days storage (Hojo et al., 2006). 'Ataulfo' mango fruit exposed to 400 nl 1-MCP, followed by storage for 7 days at 12°C and then ripen for 15 days at 20°C resulted in reduced activities of PG and cellulose, while PG activity in control fruit was the highest among others (Rangel et al., 2009).

2.6.3. Fruit quality

Mature-green mangoes cv. 'Kent' treated with 1-MCP at 0.75 $\mu l \cdot l^{-1}$ prior to storage for 14 days at 20°C showed no differences from untreated fruit in TSS and TA concentrations. This treatment was effectively retained β-carotene and ascorbic acid (AA) during storage. The concentration of β-carotene in fruit dropped to 63% and 50% in 1-MCP-treated and untreated fruit within 3 and 6 days storage period, respectively. However, β-carotene was re-synthesized and accumulated to the end of storage time. It was reported that 71% of β-carotene content increased in 1-MCP-treated fruit whilst 67% of that increased in untreated ones. Decreasing AA content during storage was monitored, but 1-MCP treatment maintained better (Islas-Osuna et al., 2010). Hot-water treated 'Kent' mango fruit exposed to 1-MCP and stored under CA (3% O_2 + 8% CO_2) showed higher hue angle value, TSS/TA ratio, ascorbic acid content, total phenolic and total flavonoid contents, and the antioxidant scavenging activity compared to those stored in 5% O_2 + 5% CO_2. This phenomenon indicated that the fruit bearing the residual effect of treatments on the fifth day of ripening. However, there were no significant differences on the quality of fruit treated with 1-MCP and CA storage (5% O_2 + 5% CO_2 or 3% O_2 + 8% CO_2) on the 10^{th} day of ripening (Sivakumar et al., 2012), suggesting that the fruit were equally ripen without any residual effect of treatments.

Wang et al. (2006) reported that 1-MCP treatment postponed the increase in AA content of 'Tommy Atkins' mango. Refrigeration followed by 1-MCP (1500 $nl \cdot l^{-1}$) treatment on 'Tommy Atkins' mango improved chroma value with gradual reduction in hue angle of the fruit skin. On the other way, when 1-MCP treatment was employed prior to refrigeration, the reduction of TA in fruit was hampered (Lima et al., 2007). The TSS and AA content were preserved better in 'Tommy Atkins' mango fruit treated with 0.6 $\mu l \cdot l^{-1}$ 1-MCP compared to control. Moreover, treated fruit retained 50% higher AA content than untreated fruit during storage at 10°C (Bomfim et al., 2011). AA content in 'Tainong' and 'Guifei' mango fruit remained high in 1-MCP-treated fruit (Wang et al., 2009; Wang et al., 2006) whereas high reduction in AA content was ascertained in C_2H_4-treated fruit (Wang et al., 2009). Skin de-greening was delayed in 'Nam Dokmai' mango fruit treated with 1-MCP during storage (Penchaiya et al., 2006). 1-MCP treatment of 'Tommy Atkins' at 100 $nl \cdot l^{-1}$ has been reported to improve pulp colour, aroma, and taste whereas 1-

MCP combined with modified atmosphere packaging (MAP) resulted in lower quality fruit (Cocozza et al., 2004a; Cocozza et al., 2004b).

The 1-MCP treatment offers great potential for extension of shelf life and to reach distant markets, especially for the climacteric produces which exhibit a fast increase in respiration and C_2H_4 production within a short period of postharvest life. The combined effect of 1-MCP and CA storage has been reported to improve the shelf life of hot-water treated 'Kent' mango fruit and ripen normally under ambient atmosphere (Sivakumar et al., 2012). However mango varieties responded differently to 1-MCP dose, length of exposure, and storage temperature. Therefore, the influence of recommended dose of 1-MCP on the Australian mango cultivar 'Kensington Pride' during storage and ripening requires investigation.

Chapter 3

General materials and methods

3.1. Fruit materials

Hard mature green mango fruit (*Mangifera indica* L. cv 'Kensington Pride') of uniform sizes, 18 or 20 fruit per tray, approx. 7.0 kg, were purchased from commercial orchards located at Chittering (long. 116°5'E, lat. 31°25'S) and Gingin (long. 113°39'E, lat. 24°52'S) in Western Australia. Fruit were packed as single – layer into cardboard trays lined with plastic inserts and transported by a refrigerated vehicle at 15 ± 1°C to the Curtin Horticultural Research Laboratory in Perth. Fruit of uniform size and, free from diseases and blemishes were used for experiments.

3.2. Chemicals

3.2.1. Chemicals for protein and enzymes analysis

Coomassie brilliant blue (G-250), 85% ortho-phosphoric acid, polygalacturonic acid, bovine serum albumin, polyethylene glycol, sodium bisulphite, sodium acetate, sodium chloride, sodium hydroxide, sodium borate, boric acid, cyanoacetamide, citrus pectin, and ethylenediamine tetra-acetic acid (EDTA) were purchased from Fluka (Sigma Aldrich, Castle Hill, New South Wales, Australia). Ethanol and acetic acid were purchased from APS (APS Ajax Finechem, Auburn, New South Wales, Australia).

3.2.2. Chemicals for skin pigment analysis

Magnesium carbonate was purchased from Fluka (Sigma Aldrich, Castle Hill New South Wales, Australia) while acetone was purchased from Mallinckrodt Chemicals (Mallinckrodt Baker Inc., Phillipsburg, New Jersey, U.S.A.).

3.2.3. Chemicals for organic acids and sugars analysis

Sulphuric acid was purchased from BDH (VWR International Ltd., Poole, U.K.). Acid and sugar standards were purchased from Sigma (Sigma Aldrich, Castle Hill, New South Wales, Australia).

3.2.4. Chemicals for other quality analysis

Magnesium carbonate, *L*-ascorbic acid standard, folin-ciocalteu's phenol reagent, sodium fluoride, sodium chloride, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carbocylic acid (Trolox), and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were purchased from Fluka and Sigma (Sigma Aldrich, Castle Hill, New South Wales, Australia). Acetone was purchased from Mallinckrodt Chemicals (Mallinckrodt Baker Inc., Phillipsburg, N.J., U.S.A.), hexane, sodium hydroxide 0.1 N, and metaphosphoric acid were purchased from BioLab (BioLab Limited, Victoria, Australia).

3.2.5. Fruit storage and ripening

As detailed in each experiment, fruit samples were stored at 13 ± 0.5°C and 85 ± 3% relative humidity and ripened at 21 ± 1°C and ambient relative humidity (58.02 ± 7.02%) until the eating soft stage. Ripeness was determined subjectively based on a firmness rating of 4 (as described in section 3.3.1) or more than 75% yellow skin (as described in section 3.5.1). Quality assessments were conducted after storage and/or ripening as detailed in each experiment.

3.3. Measurement of fruit texture

3.3.1. Non-destructive method

The subjective, non-destructive, assessment of fruit firmness was performed on individual fruit from each replication to score the firmness as described by Shorter and Joyce (1998): 1 = hard, 2 = sprung, 3 = slightly soft, 4 = eating soft, 5 = over soft.

3.3.2. Destructive methods

3.3.2.1. Fruit firmness measurement using electronic pressure tester

Firmness of the whole fruit before and after storage and/or ripening was measured on two opposite peeled surfaces in the equatorial region by electronic pressure tester (model EPT-1 pressure tester, Lake City Technical Products Inc., Kelowna, BC, Canada). The apparatus was fitted with an 11 mm-diameter plunger. The firmness was expressed in Newton.

3.3.2.2. Texture profile analysis

Texture profile analysis (TPA) of fruit pulp was performed using a Texture Analyzer (Lloyd Instruments Ltd, Farenham, Hants, U.K.). A cylindrical probe (7/16

MT) with 500 N load cell was used to measure the TPA. Fruit was cut at the equatorial region (2 cm height x 2 cm width with varying length) and peeled at the top surface (2 x 2 cm^2). Sample was than compressed in two consecutive cycles to 25% deformation at 2 $mm \cdot s^{-1}$ test speed. The textural parameters determined were hardness (N), cohesiveness, springiness (mm), gumminess (N), fracture force (N), and adhesive force (N). The typical TPA graph of mango is presented in Figure 3.1

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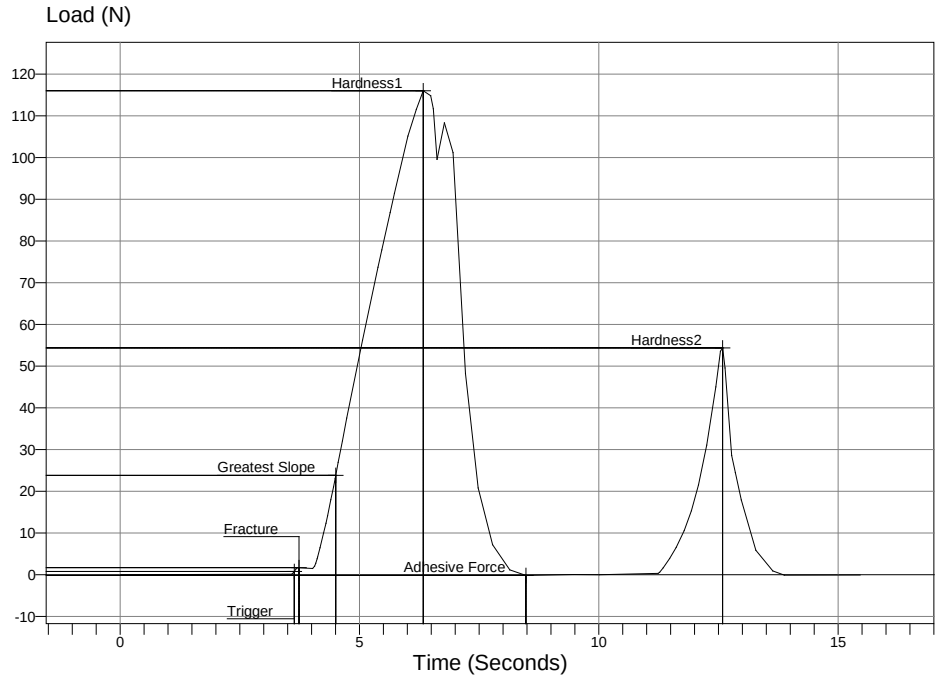

Figure 3.1. Texture profile of Kensington Pride' mango as measured by Texture Analyzer (Lloyd Instruments Ltd, Farenham, Hants, U.K.) using 500 N load cell and fitted with cylindrical probe (7/16 MT).

3.4. Determination of the activity of fruit softening enzymes

Fruit pulp softening enzymes i.e., *exo*-polygalacturonase (*exo*-PG), *endo*-polygalacturonase (*endo*-PG), and pectin esterase (PE) activities were determined according to the method of Gross (1982) and Dong et al. (2001) with some modifications. Fresh fruit pulp was stored at -80°C for enzyme analysis.

3.4.1. Extraction of *exo*-polygalacturonase (EC 3.2.1.67) and *endo*-polygalacturonase (EC 3.2.1.15)

Frozen fruit pulp tissue (13 g) was half-thawed in the cool room (4°C) and homogenised with 13 ml cold 12% polyethylene-glycol and 0.2% (w/v) sodium bisulphite in cold ceramic pestle and mortar. After centrifugation at 15,000 x g for 15 min (Eppendorf Centrifuge 5810R, Hamburg, Germany), the supernatant was discarded and the pellet was washed with 13 ml cold aqueous solution of 0.2% (w/v) sodium bisulphite and re-centrifuged at 15,000 x g for 15 min. Pellet was stored at -80°C for further extraction.

The extraction of crude enzyme was conducted following the method developed by Gross (1982) with some modifications. Frozen pellet was incubated on a shaker for 1 hour in 15 ml cold 50mM sodium acetate buffer (pH 5) solution containing 0.5 M sodium chloride. The mixture was centrifuged at 15,000 x g for 15 min, and then the supernatant was diluted 1:1 with 50 mM sodium acetate buffer (pH 5). All the extraction steps were performed at 4°C.

3.4.1.1. Determination of *exo*-polygalacturonase (EC 3.2.1.67) activity

The crude enzymes extract (0.15 ml) was mixed with 0.15 ml solution of 0.5% (w/v) polygalacturonic acid dissolved in 50 mM sodium acetate buffer (pH 4.4) and then incubated at 30°C for 18 h. The reaction was terminated by boiling (10 min) following an addition of 2 ml borate buffer (0.1 M) pH 9.0 and 0.3 ml of 1% (w/v) cyanoacetamide. The amount of galacturonic acid (GalA) released was recorded as absorbance reading at 274 nm and calculated against standard curve. The *exo*-PG activity was expressed as μg GalA released·mg protein^{-1}·h^{-1}.

3.4.1.2. Determination of e*ndo*-polygalacturonase (EC 3.2.1.15) activity

The Cannon-Fenske viscometer (Cannon Instrument Company, Pennsylvania, U.S.A.) size 50 was used to determine the activity of *endo*-PG. The crude enzymes extract (3 ml) was mixed with 4.5 ml solution of 2% polygalacturonic acid dissolved in 50 mM sodium acetate buffer (pH 4.4) and the viscosity was immediately measured. The mixture was incubated at 30°C for 18 h and then the viscosity was measured. The viscometer was cleaned with acetone and blow to dry after each run. Distilled water was used for calibration. The *endo*-polygalacturonase enzyme activity was expressed as viscosity changes in sec·mg protein^{-1}·h^{-1}.

3.4.2. Extraction and determination of the activity of pectin esterase (EC 3.1.1.11)

Frozen fruit pulp tissue (13 g) was half-thawed and passed through the same series of steps until pellet formation in the preparation of polygalacturonase enzyme extraction (Section 3.4.1.). The frozen pellet was resuspended in the 15 ml solution of 7.5% (w/v) NaCl dissolved in 0.75% (w/v) EDTA at pH 6.5. The enzyme suspension was incubated for 10 min and then centrifuged at 15,000 x g for 15 min. All steps were performed at 4°C. The supernatant, as crude enzyme extract, (5 ml) was mixed with 20 ml citrus pectin (1% w/v) and titrated against 0.01 N NaOH to maintain the pH 7.4 of the solution. The titration was conducted during incubation at 30°C for 10 min. The pectin esterase enzyme activity was expressed as mM NaOH·mg protein^{-1}·h^{-1}.

3.4.3. Estimation of protein content

Protein content from pulp tissue was determined following the method of Bradford (1976) with some modification. Coomassie brilliant blue G-250 (100 mg) was dissolved in 50 ml of 95% ethanol. One hundred ml of 85% ortho-phosphoric acid was added to the commassie brilliant blue solution. The final volume of this protein reagent was made up to 1000 ml with distilled water. Five ml protein reagent was added to 0.1 ml protein sample in 12 x 100 mm reaction tube then vortexed. The absorbance was recorded after 2 min at 595 nm using UV-VIS spectrophotometer (model 6405, Jenway Ltd., Essex, U.K.) in 2 ml disposable plastic cuvettes. A reagent blank was prepared from 0.1 ml of appropriate buffer and 5 ml protein reagent. The protein content was calculated against the standard curve of bovine serum albumin and expressed as mg·g^{-1} FW.

3.5. Fruit colour assessment

Visual and Hunter scale measurements were used in assessing the fruit skin colour. Individual fruit from each replication was evaluated during storage and/or ripening.

3.5.1. Subjective visual colour assessment

A colour rating scale was used to record the visual colour. The value was scored from 1 to 5 as follow: 1 = 100% green, 2 = 75% green, 3 = 50% green/yellow, 4 = 75% yellow, and 5 = 100% yellow (Lalel, 2002; Shorter and Joyce, 1998).

3.5.2. Spectrophotometric assessment

ColorFlex 45°/0° spectrophotometer (Hunter Associates Laboratories, Inc., Reston, Virginia, U.S.A) was used to assess the skin colour at the equatorial region of the fruit. The spectrophotometer was calibrated using black and white standard tiles. An orifice of 3 cm in diameter was fitted at the apparatus. L*, a*, and b* value were recorded which represent lightness, green/red, and blue/yellow of the fruit colour, respectively. L* value ranges from 0 (black) to 100 (white) representing the degree of lightness, a* value ranges from -60 for maximum greenness to +60 for maximum redness, and b* value represents yellow (+) or blue (-) (Figure 3.1.). The degree of saturation, expressed as chroma (C*), was then calculated from a* and b* values as $(a^{*2} + b^{*2})^{1/2}$ where low values mean dull colour and high values represent vivid colour. Hue angle (hue°), defined as a colour wheel, was calculated as $\tan^{-1}$ (b*/a*) which reflects red-purple at 0° and 360°, yellow at 90°, bluish-green at 180°, and blue at 270° (McGuire, 1992).

3.6. Skin pigment analysis

Skin pigments were determined according to Lichtenthaler (1987) with some modifications. The mango skin was removed from the fruit pulp and 0.5 g was homogenised in 20 ml of cold 80% (v/v) acetone for 2 min and centrifuged at 15,000 x g for 15 min. The chlorophylls (a, b, and total) and carotenoids from the extract were estimated by measuring the absorbance at 663.2, 646.8, 470, and 750 nm, respectively using spectrophotometer (6405 UV-VIS, Jenway Ltd., Essex, U.K.), and calculated in $\mu g \cdot g^{-1}$ FW skin. All the extraction procedure was performed in the dark room.

3.7. HPLC analysis of sugars and organic acids

High performance liquid chromatography (HPLC Waters, Milford, MA, USA) was used to determine the levels of individual and total sugars and organic acids in mango pulp. The system consisted of a Waters 717plus Autosampler conjunct to Waters 1525 Binary HPLC Pump, fitted to Waters 2487 Dual Wave length Absorbance Detector and Waters 2414 Refractive Index Detector.

The mango pulp (1 g) was homogenised in 50 ml water, centrifuged at 10,000 x g for 15 min, and the supernatant was filtered through a 0.22-µm nylon syringe-fitted filter (Alltech Associates Australia, Pty Ltd., Baulkham Hills, NSW Australia)

prior to the injection (20 µl) into the HPLC system. The water was filtered through 0.45 µm Mili-Q filter system (Millipore, Bedford, MA, USA). All mobile phases were filtered and vacuum-degassed prior to analysis.

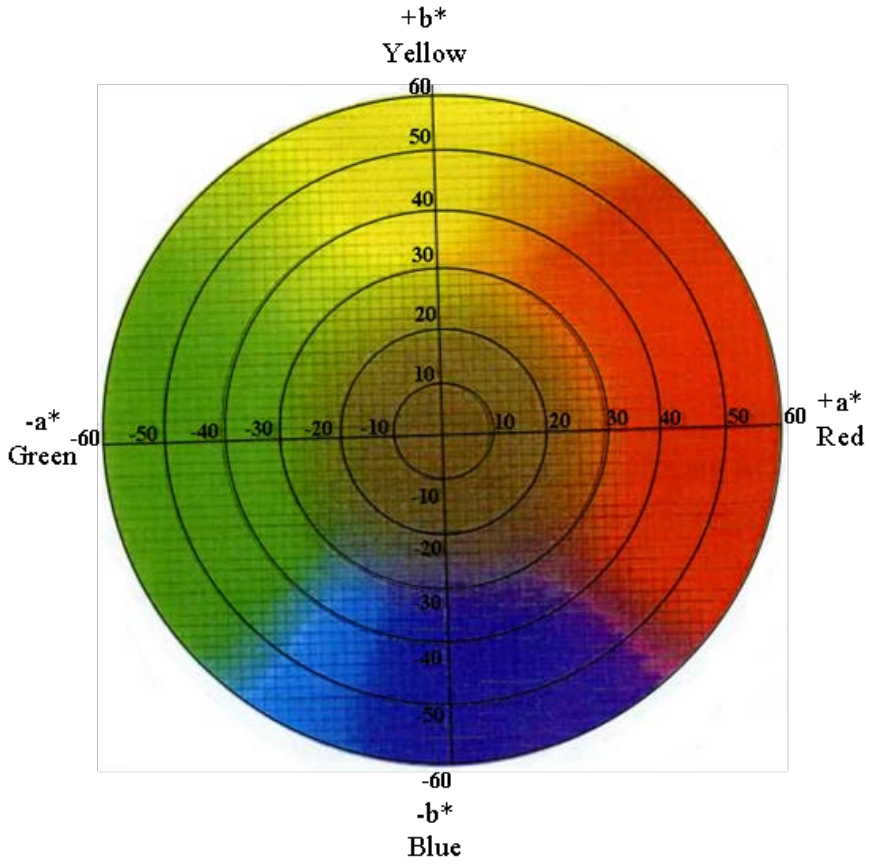

Figure 3.2. HunterLab colour chart-CIE L*, a*, b*. (Adapted from HunterLab, 1998).

3.7.1. Sugar analysis

Standards of sucrose, glucose, or fructose (0.5 g) were prepared in 100 ml water. Both standards and samples were injected through the fast carbohydrate column (100 mm x 7.8 mm, Bio Rad column) as the stationary phase with water as the mobile phase. The flow rate was 1.2 ml·min^{-1} and the elution was completed at 10 min. The column temperature was set to 60°C whilst the sample temperature was maintained to constant (25°C). The absorbance of the effluent was recorded at 410 nm (Waters 2414 Refractive Index Detector), and the individual sugar concentration was calculated base on the standard curve (R^2 = 0.9999 to 1.0000). The sugar concentration was expressed in µg·g^{-1} FW pulp.

3.7.2. Organic acids analysis

Standards of citric, malic, succinic (0.5 g) were prepared in 100 ml water. Both standards and samples were injected through the Aminex® HPX-87H ion exclusion column (300 mm x 7.8 mm) preceded by a Cation-H Bio Rad Micro-Guard column (30 x 4.6 mm) as the stationary phases with water and sulphuric acid (0.5 mM) as the mobile phases. The flow rate was 0.3 $ml \cdot min^{-1}$ (50% water and 50% sulphuric acid) and the elution time was 20 min. The column temperature was set to 45°C and the sample temperature was maintained to constant (25°C). The absorbance of the effluent was recorded at 210 nm (Waters 2487 Dual Wave length Absorbance Detector), and the individual acid concentration was calculated base on the standard curve ($R^2 = 0.9999$). The organic acid concentration was expressed in $\mu g \cdot g^{-1}$FW pulp.

3.8. Determination of total carotenoid in pulp

Mango pulp total carotenoids were estimated according to the method of Tomes (1963) and Lalel (2002). Mango pulp (2 g) and 0.05 g magnesium carbonate were homogenised twice with 20 ml of mixed solvent (75 ml acetone : 60 ml hexane) and then centrifuged at 15,000 x g (Eppendorf Centrifuge 581R, Hamburg, Germany) for 15 min. The supernatant from 2 centrifugations was combined, washed with 40 ml of 10% (w/v) sodium chloride and then with 40 ml distilled water twice. The absorbance of hexane extract was recorded at 436 nm using a spectrophotometer (6405 UV/VIS, Jenway Ltd., Essex, U.K.), calculated against standard curve of β-carotene and the concentration was expressed as $\mu g \cdot g^{-1}$ FW of pulp. The extraction was performed in the dark room.

3.9. Ascorbic acid analysis

The concentration of fruit pulp ascorbic acid was determined following the method of Jagota and Dani (1982), AOAC (1996), and Malik (2003). Mango pulp (5 g) was homogenised in 25 ml of 6% (w/v) metaphosphoric acid containing 0.18% (w/v) ethylenediamine tetra acetic acid (EDTA) disodium salt, and then centrifuged at 15,000 x g (Eppendorf Centrifuge 581R, Hamburg, Germany) for 15 min. Supernatant (400 µl) was mixed with 200 µl folin reagent (folin-ciocalteu's phenol reagent : water = 1 : 5 v/v), 200 µl of 3% meta-phosphoric acid, and 1,400 µl distilled water for an assay. The absorbance at 760 nm was recorded by spectrophotometer (6405 UV-Vis, Jenway Ltd., Essex, U.K.). The quantification of ascorbic acid in

mango pulp was based on the standard curve of *L*-ascorbic acid and expressed as mg ascorbic acid per 100 g FW pulp

3.10. Estimation of total antioxidants

Total antioxidant activity was determined following the method of Brand-Williams et al. (1995) with some modifications. The extraction buffer (2 mM sodium fluoride) was prepared by dissolving 84 mg of sodium fluoride in 200 ml distilled water and diluted to 1,000 ml with methanol. The stock solution of 2,2-diphynyl-1-picrylhydrazyl (DPPH) was prepared by dissolving 21 mg DPPH in 100 ml methanol. This stock solution is stored in refrigerator for less than 5 days. The stock solution was further diluted with methanol (1:4, v/v) to give the reading of 1.1 at 515 nm (spectrometer 6405 UV-Vis, Jenway Ltd., Essex, U.K.) for daily analysis. Three g mango pulp was homogenised in 10 ml extraction buffer and centrifuged at 15,000 x g (Eppendorf Centrifuge 581R, Hamburg, Germany) for 15 min. The supernatant (15 µl) was mixed with 950 µl daily solution and the absorbance between 0.6 - 0.7 was recorded after 15 min. Diluted or concentrated sample was prepared when the absorbance reading was beyond the range. The total antioxidant was calculated against standard curve of 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and expressed as µmol Trolox Equivalent (TE)·100 g^{-1} FW pulp.

3.11. Statistical analysis

The data were assessed within one-way or two-way analysis of variance (ANOVA) depending upon experiment using Genstat 9 release 9.1.0.147 (Lawes Agricultural Trust, Rothamsted Experimental Station , Rothamsted, U.K.). The effects of various treatments and their interactions on various parameters were assessed within ANOVA and least significant differences (Fisher's protected LSD) were calculated at $P \leq 0.05$ following a significant F test. The validity of analysis was confirmed by checking all the assumptions of analysis.

Chapter 4

Effects of atmosphere composition on softening, colour development, and quality during storage and ripening of mango fruit

Summary

The objective was to study the influences of storage atmosphere composition on fruit softening, colour development, and quality during storage and ripening in 'Kensington Pride' mango fruit. In the first experiment, 'Kensington Pride' mangoes at the hard mature green stage were harvested and stored in normal air (NA) at 13 ± 0.5°C or in CA chambers containing 3% CO_2 with 4%, 5%, or 6% CO_2 (balance N_2) at 13 ± 0.5°C and 85 ± 3% RH for 4 weeks and transferred to ambient conditions at weekly intervals. In the second experiment, fruit were stored under same conditions as in experiment 1, but were removed after 4 weeks of storage and allowed to ripen at 21 ± 1°C until eating soft stage.

CA maintained the fruit firmness better than NA up to 4 weeks storage; however, CA conditions did not effectively retard the activity of *exo*- and *endo*-polygalacturonase, and pectin esterase. CA conditions delayed the fruit skin colour development together with the retardation of chlorophylls degradation for up to 4 weeks, although skin carotenoids synthesis was measured only within 3 weeks CA storage. Sugar and acid concentrations were higher in CA-stored fruit as compared to NA-stored fruit after 4 weeks storage. In all CA-stored fruit pulp tissue, lower carotenoids levels, and higher ascorbic acid concentrations were observed as compared to NA-stored fruit.

During ripening, fruit stored for 4 weeks in CA were firmer compared to those stored in NA. The increase in *exo*-PG activity of NA-stored fruit was delayed until day 3 of ripening whilst that of CA-stored fruit started at day 1 and 2. The *exo*-PG activity in ripe fruit stored in CA was higher than NA-stored fruit as opposed to *endo*-PG activity in CA-stored ripe fruit, which was lower. PE activity was delayed in NA-stored, but not in CA-stored fruit. Furthermore, ripe fruit from CA storage (3% O_2 combined with 5% or 6% CO_2) had higher PE activity compared to ripe fruit from NA and CA storage comprised of 3% O_2 with 4% CO_2. Low skin colour development and

chlorophylls degradation suggest residual effects of CA storage during ripening. The skin carotenoids of CA-stored fruit degraded in higher rate compared to those at a NA-stored fruit. After 4 weeks of storage, pulp tissues of CA-stored fruit had higher contents of sugars (e.g. sucrose, glucose, and fructose) and acids (i.e. citric, succinic, and ascorbic), but lower carotenoids content compared to NA-stored fruit, where as total antioxidant activity was not affected.

In conclusion, CA storage effectively delayed loss of fruit firmness, colour development, and chlorophylls degradation in fruit skin; it also delayed carotenoids synthesis, both in the fruit skin and pulp tissue during storage and ripening of 'Kensington Pride' mango fruit. CA storage also resulted in high sugars and organic acids levels of mango pulp tissue at 4 weeks of storage, but low sugars accompanied with high acids at 4 days of ripening. CA storage effectively retarded *exo*- and *endo*-PG activity up to 3 weeks, and PE activity up to 4 weeks of storage. However, CA storage did not delay PG and PE activities during 4 days of fruit ripening. CA storage maintained ascorbic acid content in fruit pulp tissue during ripening.

4.1. Introduction

Mango fruit has short postharvest life depending upon cultivar, harvest maturity, postharvest handling and storage conditions. Various postharvest handling techniques have resulted in limited success in extending storage life, delaying ripening, and reducing the postharvest losses of mango fruit. Application of polyamines (Gonzalez-Aguilar et al., 2001; Malik, 2003; Martínez-Romero et al., 2002), 1-MCP, AVG (Lalel, 2002), edible coatings (Castrillo et al., 1992; Dang et al., 2008a; DiazSobac et al., 1997; Hoa and Ducamp, 2008), low temperature storage (Nair et al., 2004a, b), modified atmosphere packaging (Illeperuma and Jayasuriya, 2002; Tefera et al., 2007; Yahia, 1997), and controlled atmosphere storage (Bender and Brecht, 2000; Lalel et al., 2001; Lalel et al., 2003a; Lalel et al., 2006; Singh and Zaharah, 2015; Yahia and Ortega-Zaleta, 2000) are some of the postharvest techniques for handling mango. Reduced oxygen (Teixeira and Durigan, 2011) and elevated carbon dioxide during storage of fresh horticultural products can either induce or depress the metabolic activities, depending on the exposure, nature, and the suitability of the produce to the CA condition (Beaudry, 1999; Kader, 2003a; Mathooko, 1996a).

The air composition during CA storage of mango is considered to be the critical factor affecting its storage life and quality. CA has been reported to extend storage life and maintain fruit quality in different mango cultivars such as 'Irwin' (Maekawa, 1990), 'Tommy Atkins' (Abdulah and Basiouny, 2000; Bender et al., 2000a; Kim et al., 2007; Lizana and Ochagavia, 1996), 'Haden' (Bender et al., 2000b), 'Keitt' (Gonzalez-Aguilar et al., 1997; Yahia and Hernandez, 1993), 'Kent' (Bender et al., 2000b; Lizana and Ochagavia, 1996), 'Rad' (Noomhorm and Tiasuwan, 1995), R2E2 (Lalel et al., 2006), 'Kensington Pride' (Dang et al., 2008b; Lalel et al., 2001; Lalel et al., 2003a; McLauchlan and Barker, 1992), and 'Palmer' (Teixeira and Durigan, 2011). Storage atmosphere composition comprising of 3% oxygen and 6% carbon dioxide has been commercially applied for exporting 'Kensington Pride' mango from Australia to United Kingdom by sea freight (Anonymous, 2002; Lalel, 2002). However, tissue injury, fruit softening, poor colour, and off-flavour developments in ripe mango fruit are listed as problems that arise following CA storage (Anonymous, 2002; Beaudry, 1999; Bender et al., 2000a; Bender et al., 2000b; Dang et al., 2008b; Lalel et al., 2006).

Controlled atmosphere storage have been proved to effectively maintain firmness and green colour of 'Tommy Atkins', 'Keitt' (Brecht et al., 2003), 'Alphonso' and 'Banganapalli' (Rao and Rao, 2008) mango cultivars. Improved fruit firmness was also reported by Lalel et al. (2003a) when 'Kensington Pride' mango fruit were stored in 3% oxygen and 6% carbon dioxide for 7 weeks in CA storage. The green colour persists when mango stored under CA condition has been reported (Kim et al., 2007; Lalel et al., 2001). 'Palmer' mango was reported to ripe normally within 8 days after low-oxygen storage (Teixeira and Durigan, 2011). On the other hand, 'Alphonso' and 'Banganapalli' mangoes ripen under normal condition after different CA storage exhibited various degree of firmness, colour development, and sugar content regardless of their cultivar (Rao and Rao, 2008). Yahia and Vazquez-Moreno (1993) also reported that CA storage (2% O_2 and 50% CO_2) interfers the activity of glycolytic enzymes during ripening of 'Keitt' mango. Most of the research work has been reported on the effects of CA storage on extending storage life and quality of mango fruit. No research work has been reported on the effects of the gas composition in CA storage on softening mechanism, colour changes, and nutritive value of 'Kensington Pride' during storage and ripening. The aim of this study was to

investigate the effects of different atmosphere compositions on fruit firmness, softening enzymes activities, colour development, pigmentations, sugars and organic acids levels, and nutritive value during storage and ripening of 'Kensington Pride' mango.

4.2. Materials and methods

4.2.1. Fruit and experimental conditions

4.2.1.1. *Experiment 1*: Controlled atmosphere storage of 'Kensington Pride' mango

Hard mature green 'Kensington Pride' mangoes were sourced from a commercial orchard located at Chittering, Western Australia in March 2006. After harvest and de-sapping, fruit were dipped for two minutes in an aqueous fungicide solution containing 0.55 $ml \cdot l^{-1}$ 'Sportak' (prochloraz as an active ingredient), air dried, packed, and transported to Perth, as described in section 3.1. Uniformly mature fruit (fruit firmness 127.26 ± 1.53 N and respiration rate 0.98 ± 0.25 mmol $CO_2 \cdot kg^{-1} \cdot h^{-1}$), free from visual symptoms of any diseases or blemishes were stored in normal atmosphere (NA) and in 90-litre chambers of controlled atmosphere (CA) containing 3% O_2 and 4% CO_2 (CA1), 3% O_2 and 5% CO_2 (CA2), and 3% O_2 and 6% CO_2 (CA3) at 13 ± 0.5°C and 85 ± 3% RH. Concentration of O_2 and CO_2 in the CA chambers were adjusted with N_2. The CA storage was a continuous gas flow with open ended system, and the conditions were maintained and monitored by a Gas Analyser (ADC 7000 series, Analytical Development Company Ltd., Hoddesdon, Herts, UK). A single chamber containing 10 fruit was treated as one experimental unit and replicated three times, 12 chambers were used. The fruit were removed after 1, 2, 3, and 4 weeks of storage.

4.2.1.2. *Experiment 2:* The influence of controlled atmosphere storage on ripening of 'Kensington Pride' mango

Hard mature green 'Kensington Pride' mangoes (fruit firmness 125.09 ± 3.37 N and respiration rate 0.98 ± 0.15 mmol $CO_2 \cdot kg^{-1} \cdot h^{-1}$) were sourced from the same location as experiment 1 in April 2006. The fruit were treated same as the first experiment until 4 weeks in storage, than removed and ripened as described in section 3.2.5. Fruit were assessed for parameters described below at removal from 4 weeks storage, than at 1, 2, 3, and 4 days afterwards.

Both experiments were laid out by following a completely randomised design with 10 fruit as an experimental unit and three replications.

4.2.2. Measurement of fruit firmness

Firmness of the whole fruit before and after storage and/or at ripening was measured on two opposite peeled surfaces in the equatorial region by electronic pressure tester as described in Chapter 3 section 3.3.2.1.

4.2.3. Fruit softening enzyme activities analysis

The activity of fruit pulp softening enzymes i.e., *exo*-polygalacturonase (*exo*-PG), *endo*-polygalacturonase (*endo*-PG), and pectin esterase (PE) were determined. The extraction and analysis of *exo*- and *endo*-PG activities were described in section 3.4.1. The extraction and analysis of PE activities were described in section 3.4.2. The protein analysis of mango pulp was described in section 3.4.3.

4.2.4. Fruit colour assessment

Spectrophotometer and visual measurements were used to assess fruit skin colour. Individual fruit from each replication was evaluated during storage and/or ripening, as described in section 3.5. The chromaticity of the fruit skin were assessed in L*, a*, and b* values, followed by calculations for the chroma and hue angle. The visual evaluation was conducted as described in section 3.5.1.

4.2.5. Skin pigment analysis

Skin pigments (chlorophylls and carotenoids) were measured as described in section 3.6.

4.2.6. HPLC analysis of sugars and organic acids

High performance liquid chromatography was used to determine the contents of sugars and organic acids as detailed in section 3.7.

4.2.7. Pulp ascorbic acid analysis

The concentration of fruit pulp ascorbic acid was determined as detailed in section 3.9.

4.2.8. Pulp total carotenoid analysis

Mango pulp total carotenoids were estimated as described in section 3.8.

4.2.9. Determination of total antioxidants

Total antioxidant was determined as detailed in section 3.10.

4.2.10. Statistical analysis

Effects of different atmosphere during storage and ripening on fruit softening, colour changes, and nutritive value of 'Kensington Pride' mango were assessed within two-way ANOVA as described in section 3.11.

4.3. Results

4.3.1. *Experiment 1*: Controlled atmosphere storage of 'Kensington Pride' mango

4.3.1.1. Fruit firmness

Different storage atmospheres and period significantly ($P \leq 0.05$) affected fruit firmness (Table 4.1 and 4.2). The interaction between storage atmosphere and period also significantly affected fruit firmness. Fruit stored in CA (3% O_2 combined with 4, 5, and 6% CO_2) were significantly firmer than those stored in NA at each weekly storage interval, especially after 1 weeks of storage (Figure 4.1). During storage in NA, fruit firmness decreased by about 50% within the first two weeks and further decreased by an additional 40% during weeks 3 and 4. The differences in fruit firmness at weeks 3 and 4 were not statistically significant. Irrespective of CA conditions, longer storage duration resulted in decreased fruit firmness. Fruit stored in CA2 (3% O_2 + 5% CO_2) showed significantly higher fruit firmness (50.7 N) after 4 weeks storage compared to those stored in CA1 (3% O_2 + 4% CO_2) and CA3 (3% O_2 + 6% CO_2) i.e., 37.3 N and 37.6 N, respectively.

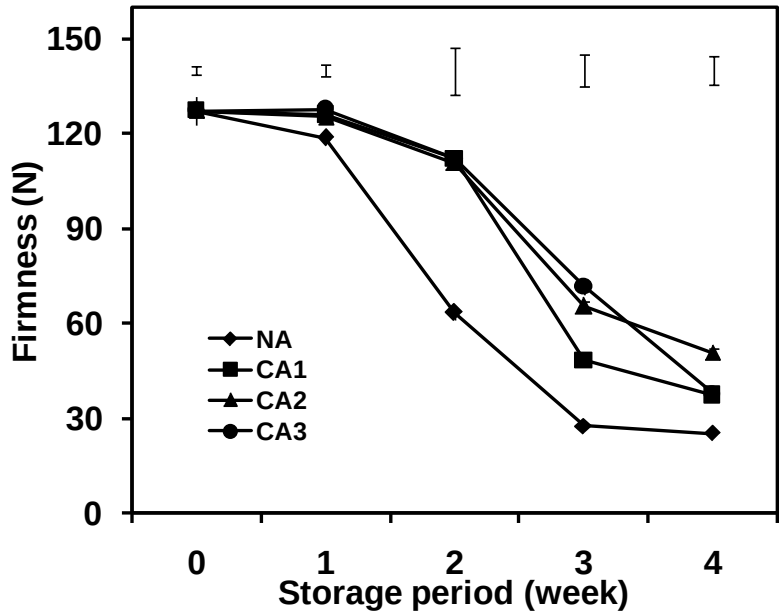

Figure 4.1. Effects of different levels of CO_2 in storage atmosphere (SA) on firmness of mango fruit during storage period (SP). Vertical bars out side graphs represent LSD ($P \leq 0.05$). LSD ($P \leq 0.05$): SA = 3.59, SP = 4.02, SA x SP = 8.04. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2.

4.3.1.2. Fruit softening enzyme activities

4.3.1.2.1. Exo- and endo-polygalacturonase enzyme activities

The activities of *exo*- and *endo*-polygalacturonase enzymes in the fruit pulp were significantly affected by storage atmosphere and storage period ($P \leq 0.05$) (Figure 4.2). The interaction between storage atmosphere and period were found to be significant ($P \leq 0.05$) for both enzyme activities. CA-stored fruit exhibited significantly lower *exo*-polygalacturonase (*exo*-PG) activity than those held in NA for 3 weeks, but its activity was either higher or similar after 4 weeks of storage (Figure 4.2A). After 4 weeks storage, the highest *exo*-PG activity was observed in fruit under CA3 which was about three folds higher than those in CA2 and NA, and 1.6 folds higher than those in CA1.

The activity of *endo*-polygalacturonase (*endo*-PG) increased significantly during the first week of NA storage, declined subsequently in week 2, and then remained almost constant during weeks 3 and 4 (Figure 4.2). Contrarily, *endo*-PG

activity in CA-stored fruit increased significantly in week 4. The highest *endo*-PG activity was recorded in fruit stored under CA3 which was 2.7, 1.5, and 5.6 folds higher than those in CA1, CA2, and NA, respectively.

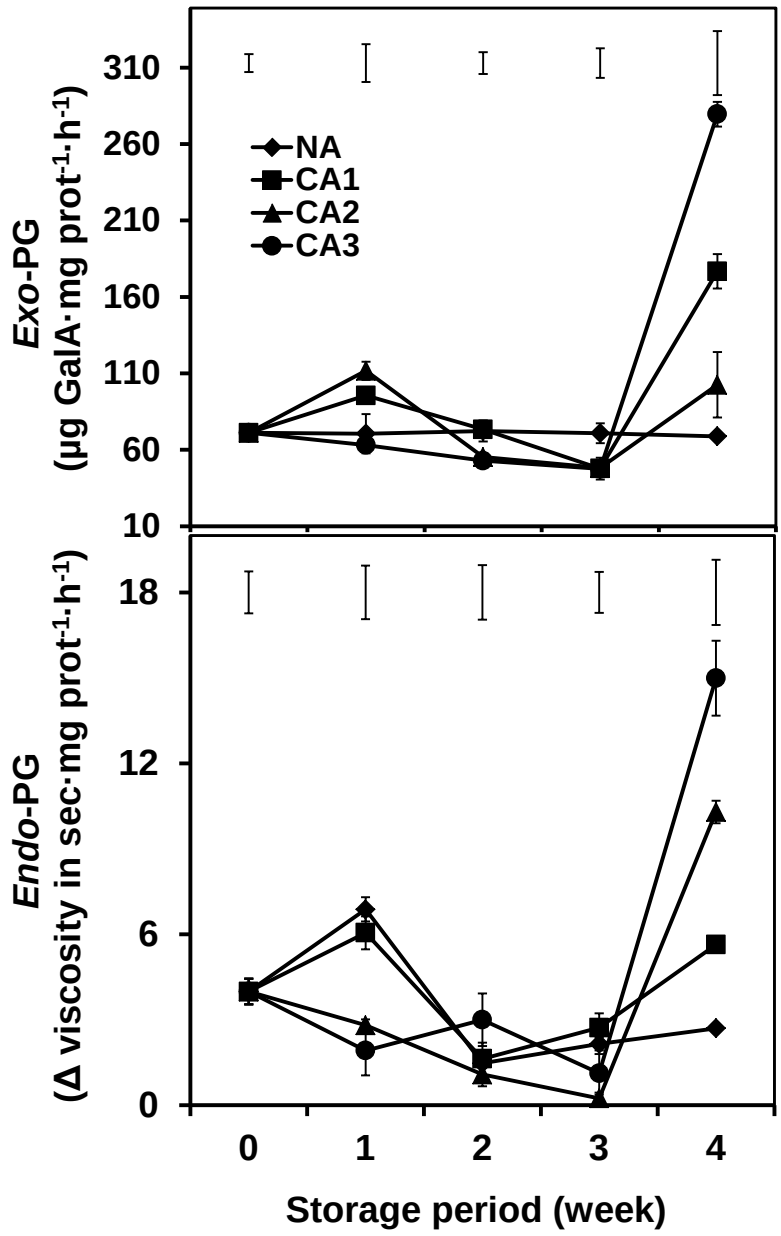

Figure 4.2. Effects of different storage atmospheres (SA) and storage period (SP) on *exo*- and *endo*-polygalacturonase enzyme activity of mango fruit pulp during storage. Vertical bars out side graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$) for (A) *exo*-polygalacturonase enzyme activity: SA = 9.37, SP = 10.88, SA x SP = 21.76; (B) *endo*-polygalacturonase enzyme activity: SA = 0.72, SP = 0.80, SA x SP = 1.60. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2.

4.3.1.2.2. Pectin esterase enzyme activity

Storage atmospheres and storage period significantly ($P \leq 0.05$) affected the activity of pectin esterase (PE) enzyme in mango fruit pulp (Figure 4.3). The interaction between storage atmospheres and storage period was also significantly ($P \leq 0.05$) affected the PE activity. All CA-stored fruit showed significantly higher PE activity than those in NA storage after 1 to 3 weeks, and either higher or similar after 4 weeks. PE activity in fruit pulp decreased significantly (44%) after 1 week of storage in NA, and 2 weeks of storage in CA (57% and 53% in CA2 and CA3, respectively) except fruit from CA1 which increased 23%. No significant decrease in PE activities was observed after 2 to 4 weeks in NA-stored, and 3 to 4 weeks in all CA-stored fruit.

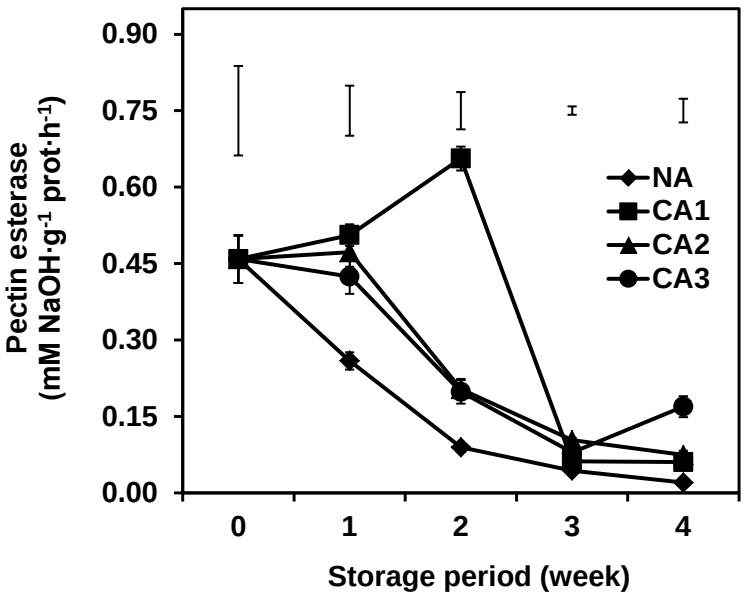

Figure 4.3. Effects of different storage atmospheres (SA) and storage period (SP) on pectin esterase enzyme activity in mango fruit pulp during storage. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$): SA = 0.01, SP = 0.01, SA x SP = 0.02. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2.

4.3.1.3.Fruit colour

Fruit skin colour was significantly ($P \leq 0.05$) affected by storage atmospheres and storage period (Figure 4.4). Fruit colour lightness (L*), a*, b*, and saturation (C*) significantly improved during 4 weeks storage in NA but not for those in CA. After 2 weeks in NA storage, fruit exhibited lower hue angle whereas no significant changes were noticed in CA-stored fruit throughout the storage period. Similarly, the visual colour of NA-stored fruit developed more yellow than all CA-stored fruit during 4 weeks storage.

4.3.1.4.Fruit skin pigments

The chlorophylls level in mango skin was significantly ($P \leq 0.05$) influenced by storage atmospheres and storage period. The interaction between storage atmospheres and storage period was also significantly ($P \leq 0.05$) affected the level of chlorophylls in fruit skin. NA-stored fruit lost more than 50% of total chlorophylls compared to less than 40% in all CA-stored fruit after 4 weeks (Figure 4.5). Among CA-stored fruit, the CA3-stored fruit generally retained higher chlorophylls contents (116.07 $\mu g \cdot g^{-1}$ FW), although there were no significant differences at the end of storage.

As the chlorophylls degraded, the carotenoids content significantly increased during storage. However, different storage atmospheres did not significantly ($P \leq 0.05$) affect the carotenoids levels in mango fruit skin. The interaction between storage atmosphere and storage period did not significantly affect carotenoids synthesis in fruit skin (Figure 4.5).

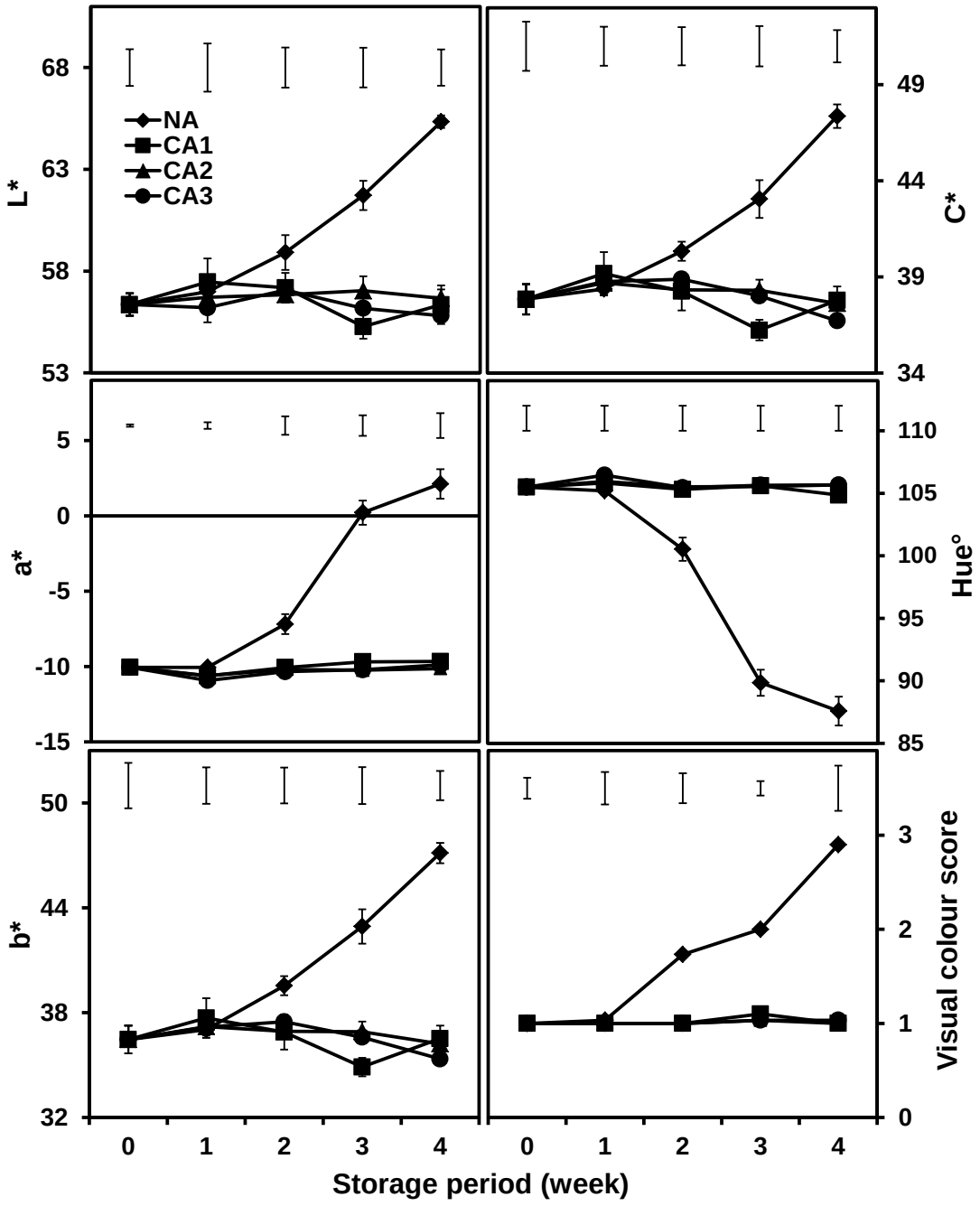

Figure 4.4. Effects of different storage atmospheres (SA) and storage period (SP) on skin colour of mango fruit during storage. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 30, 3 replications with 10 fruits in each replication). LSD ($P \leq 0.05$) for L*: SA = 0.90, SP = 1.00, SA x SP = 2.00; a*: SA = 0.47, SP = 0.52, SA x SP = 1.04; b*: SA = 1.02, SP = 1.13, SA x SP = 2.27; chroma: SA = 1.00, SP = ns, SA x SP = 2.24; Hue°: SA = 0.67, SP = 0.74, SA x SP = 1.49; visual colour: SA = 0.15, SP = 0.17, SA x SP = 0.33. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2.

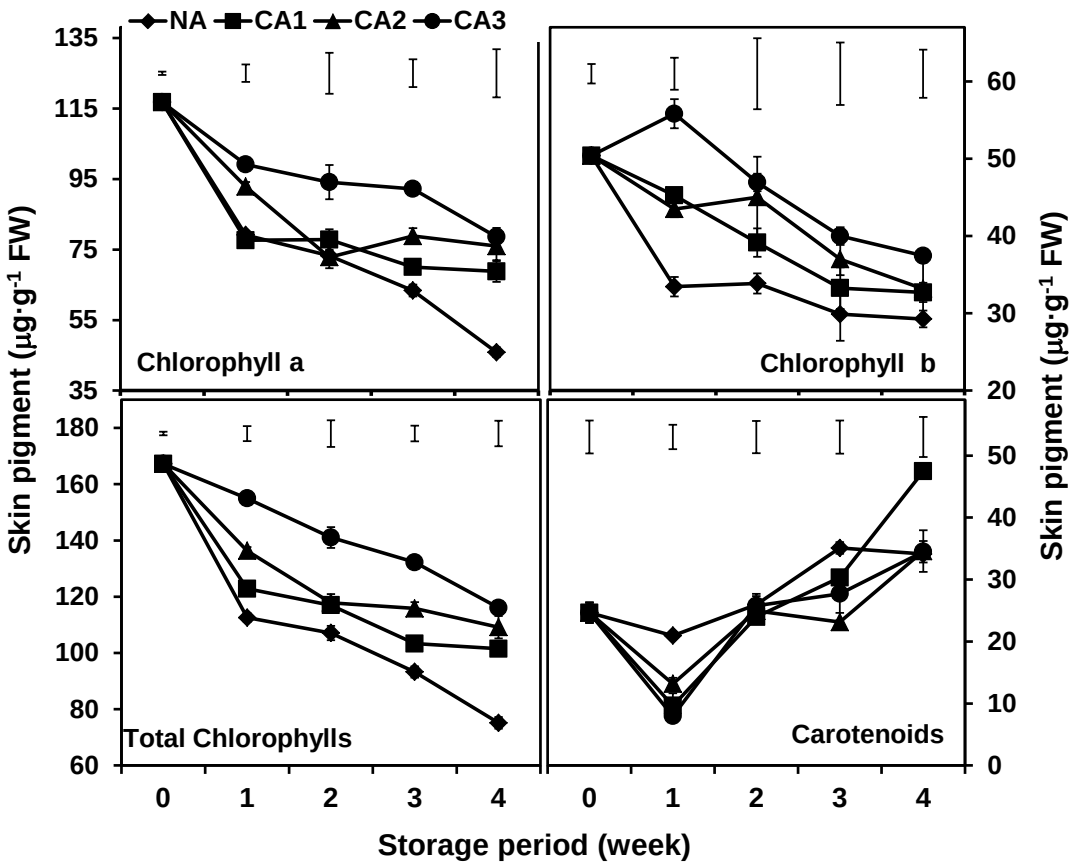

Figure 4.5. Effects of different storage atmospheres (SA) and storage period (SP) on skin pigments of mango fruit during storage. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$) for chlorophyll a: SA = 8.83, SP = 9.87, SA x SP = 19.74; chlorophyll b: SA= 5.44, SP = ns, SA*SP = ns; total chlorophylls: SA = 13.21, SP = 14.77, SA x SP = 29.53; carotenoids: SA = ns, SP = 3.03, SA x SP = ns. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2, ns = not significant.

4.3.1.5.Sugars and organic acids

4.3.1.5.1. Sugars

The effects of different storage atmospheres and period of storage were significant ($P \leq 0.05$) on sucrose, glucose and fructose levels in mango fruit pulp (Figure 4.6). The sucrose levels increased significantly until week 3 in storage then remained stable in CA2- and CA3-stored fruit, whereas declined in NA and CA1 treated fruit. NA-stored fruit showed significantly lower (28.75 µg·mg^{-1} FW) sucrose content compared to all CA-stored fruit (49.77, 46.58, and 46.82 µg·mg^{-1} FW in CA1,

CA2, and CA3 treated fruit, respectively) after 4 weeks. Glucose increased significantly in all CA-stored fruit until week 4, but only until week 2 in NA-stored fruit before it decreased. No significant differences were detected in glucose content of NA-stored and CA-stored fruit until 2 weeks storage. Significantly lower glucose content was noticed at 3 and 4 weeks storage in NA-stored compared to that in all CA-stored fruit.

Changes in fructose concentrations were noticed during storage of NA- and all CA-stored fruit until 3 weeks. Further storage reduced fructose levels in NA and CA1 stored fruit but not in CA2 and CA3 stored fruit. The fruit stored in CA1 had higher fructose compared to those stored in NA and other CA conditions at week 1. No significant difference among NA, CA1, and CA3, but lower fructose content in CA2 at week 1, although it was not significantly different from other CA treatments at week 2. NA and CA1 showed higher fructose levels than CA2 and CA3 treated fruit at week 3. However, fructose levels in NA-stored fruit (14.80 $\mu g \cdot mg^{-1}$ FW) were lower than all CA-stored fruit (26.67, 27.64, and 27.07 $\mu g \cdot mg^{-1}$ FW for CA1, CA2, and CA3, respectively) after 4 weeks storage.

4.3.1.5.2. Organic acids

Storage atmosphere conditions and time significantly ($P \leq 0.05$) influenced the organic acids levels in fruit, except malic acid. The interaction between storage atmosphere and storage period was significant at $P \leq 0.05$. The citric acid attained its peak at week 2 in storage regardless of the storage conditions (Figure 4.7), significantly lower in CA1-stored compared to NA-stored fruit but no significant difference among CA treatments. Except fruit stored in CA1, citric acid levels in all other storage atmosphere treatment declined significantly during 4 weeks storage. NA-stored fruit lost the highest citric acid concentrations (82.4%), followed by CA3 (36.22%), and then CA2 (23.61%) stored fruit.

CA treatments did not exhibit significant influence on the reduction of malic acid levels during storage. However, they showed significantly higher malic acid levels compared to NA treatment after 4 weeks storage. Succinic acid levels increased significantly in the fruit stored under NA and CA3 conditions in contrast to the decreasing phenomenon in fruit stored under CA1 and CA2 until week 2. These contradictory circumstances occurred in reverse trend at week 3 forward. The highest

reduction in succinic acid levels occurred in NA-stored fruit (68.12%), and the highest increase in this acid level was in CA2-stored fruit (33.44%) although the difference between CA1 and CA2 was non-significant (Figure 4.7).

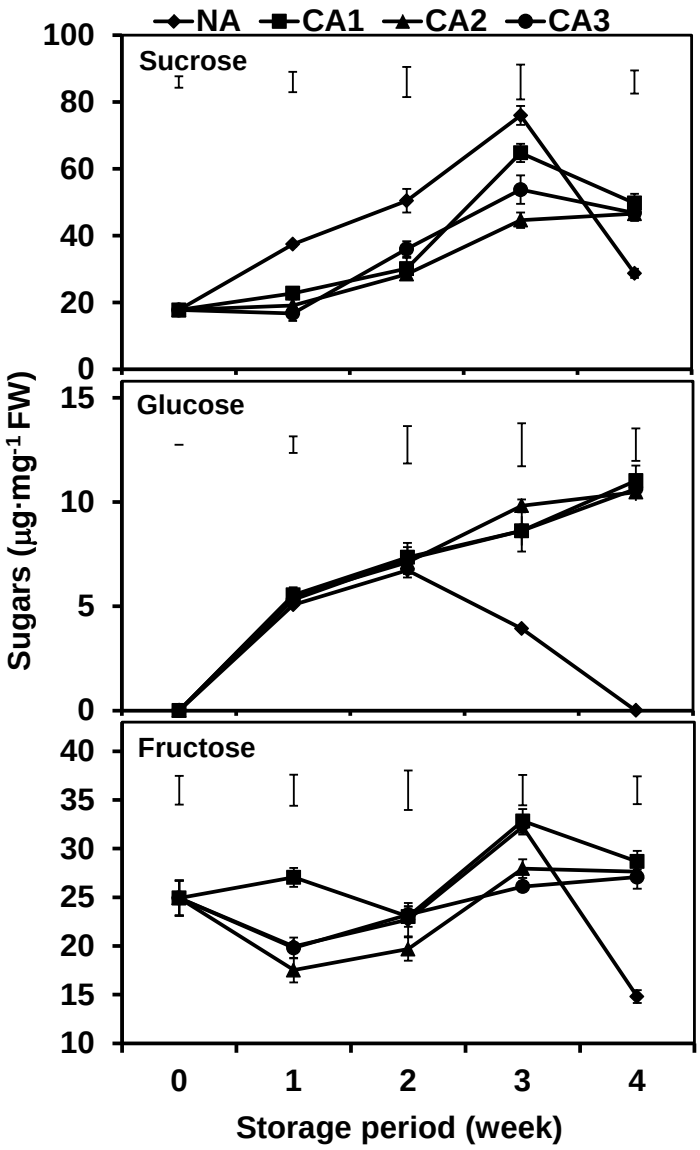

Figure 4.6. Effects of storage atmospheres (SA) and storage period (SP) on sugar levels in mango fruit pulp during storage. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$) for sucrose: SA = 2.97, SP = 3.32, SA x SP = 6.64; glucose: SA= 0.57, SP = 0.64, SA x SP = 1.27; fructose: SA = 1.28, SP = 1.43, SA x SP = 2.86. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2.

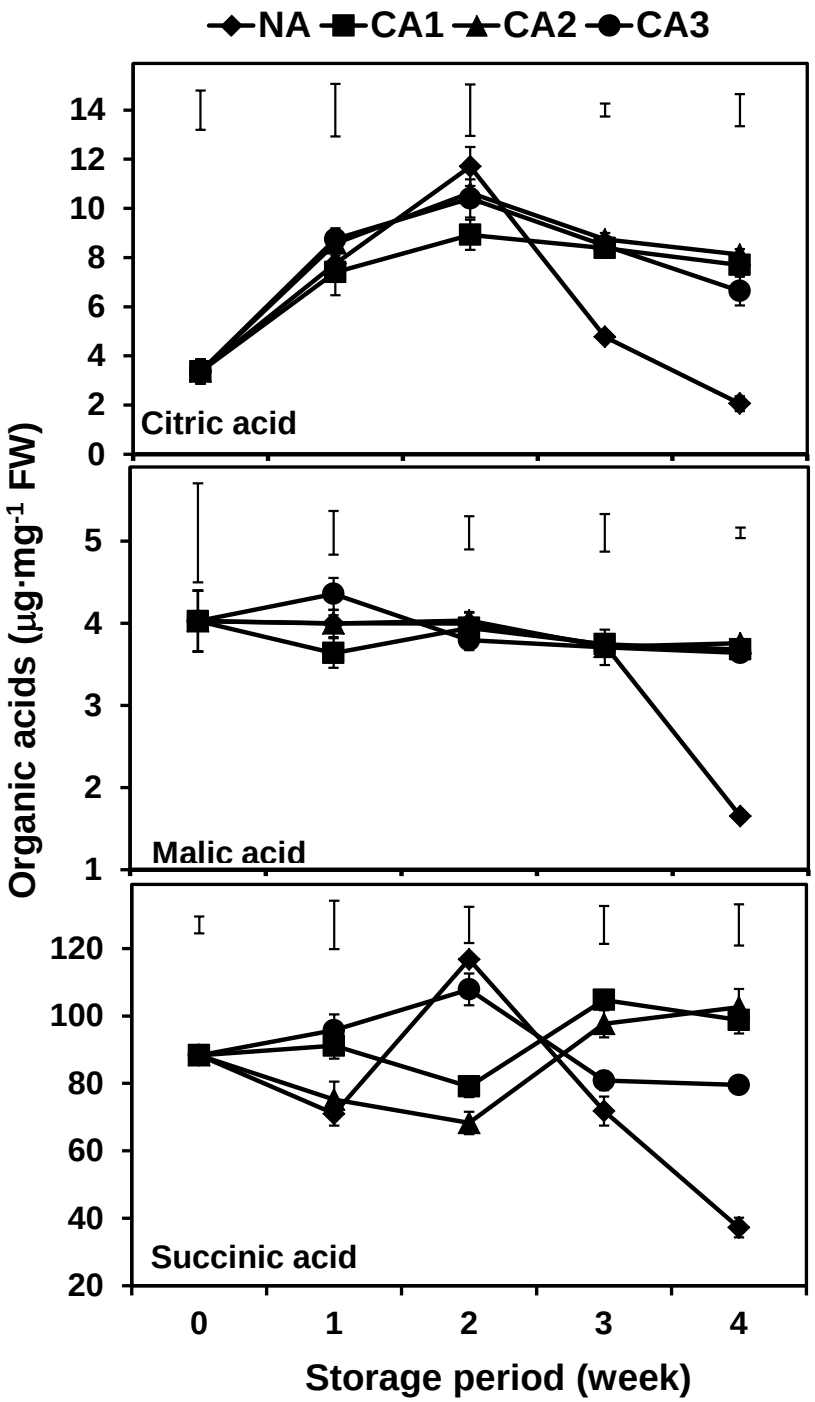

Figure 4.7. Effects of storage atmospheres (SA) and storage period (SP) on organic acid levels in mango fruit pulp during storage. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$) for citric acid: SA = 0.64, SP = 0.72, SA x SP = 1.44; malic acid: SA= ns, SP = 0.29, SA x SP = 0.57; succinic: SA = 4.39, SP = 4.90, SA x SP = 9.81. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2 ns = not significant.

4.3.1.6.Carotenoids, ascorbic acid, and total antioxidants in mango pulp

Different storage atmospheres and storage time significantly ($P \leq 0.05$) affected the levels of carotenoids in mango pulp (Table 4.1 and 4.2). The interaction between storage atmospheres and storage time significantly ($P \leq 0.05$) influenced the carotenoids levels in mango pulp. All storage conditions showed increasing carotenoids levels at week 3 (377.78%, 37.00%, 25.94%, and 29.78% in NA-, CA1-, CA2-, and CA3-stored fruit, respectively). The carotenoids levels in all CA-stored fruit was significantly lower than that stored in NA, but no significant difference was noticed among CA-stored fruit at week 3. Following 4 weeks storage, carotenoids levels in NA-stored fruit remained high compared to all CA-stored fruit. The fruit stored in CA1 and CA3 exhibited the lowest carotenoids levels and significantly low compared to CA2-stored fruit (Figure 4.8).

Storage atmospheres did not have significant effect on ascorbic acid content. However, the storage period significantly ($P \leq 0.05$) influenced the ascorbic acid levels in fruit pulp tissue. At week 4, all CA-stored fruit contained significantly higher (32%) ascorbic acid than NA-stored fruit (Figure 4.9). The interaction between storage atmospheres and storage period significantly ($P \leq 0.05$) influenced the ascorbic acid levels.

Significant effects of storage atmospheres and storage duration were noticed on the total antioxidants of 'Kensington Pride' mango pulp. The interaction between storage atmospheres and storage period significantly ($P \leq 0.05$) influenced the total antioxidant activity (Figure 4.10). At week 3, total antioxidant activity decreased significantly for most treatments except CA1. At week 4, total antioxidant activity in fruit stored in CA1 (3.20 μmol TE·100 g FW) was higher than all other treatments.

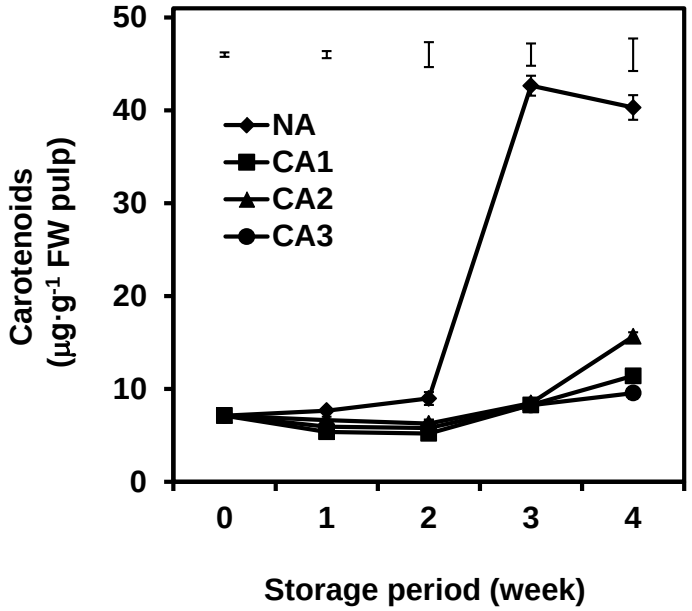

Figure 4.8. Effects of storage atmospheres (SA) and storage period (SP) on carotenoids of mango fruit pulp during storage. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$): SA = 0.90, SP = 1.00, SA x SP = 2.01. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2.

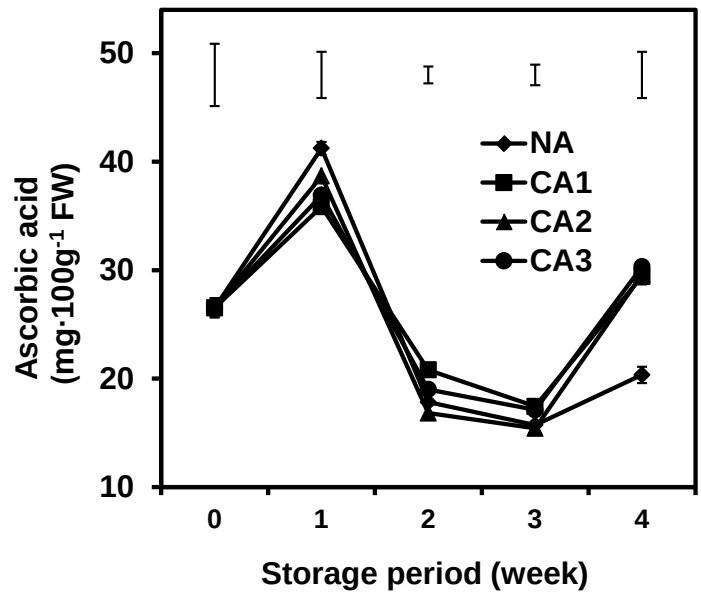

Figure 4.9. Effects of storage atmospheres (SA) and storage period (SP) on ascorbic acid of mango fruit pulp during storage. Vertical bars out side graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$): SA = ns, SP = 1.70, SA x SP = 3.40. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2, ns = not significant.

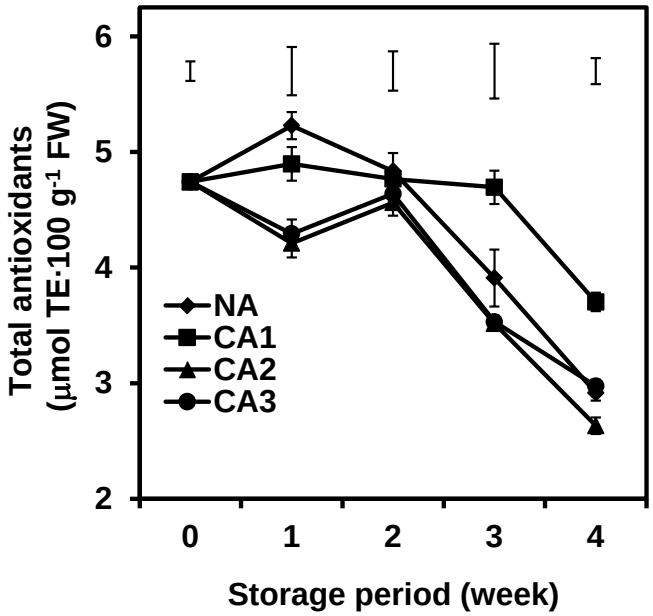

Figure 4.10. Effects of storage atmospheres (SA) and storage period (SP) on total antioxidant activity of mango fruit pulp during storage. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$): SA = 0.14, SP = 0.15, SA x SP = 0.30. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2.

The mean values of parameters measured during 4 weeks of storage of 'Kensington Pride' mango are presented in Table 4.1 and 4.2. Storage conditions (i.e. the average of all 5 removals; Table 4.1) affected most parameters except skin carotenoids, malic acid, and ascorbic acid. Likewise, storage duration (i.e. the average of all 4 treatments; Table 4.2) affected most parameters except chroma and chlorophyll b.

Table 4.1. Experiment 1: Mean firmness, activities of *exo*-PG, *endo*-PG , and PE enzymes, L*, a*, b*, chroma, hue°, visual colour, chlorophylls (a, b, and total), carotenoids (skin and pulp), sugars, organic acids, ascorbic acid, and total antioxidants in mango fruit influenced by different storage atmospheres at 13°C

Parameters	Storage atmospheres				LSD ($P \leq 0.05$)
	NA	CA1	CA2	CA3	
Firmness (N)	72.37	90.16	95.94	95.21	3.59
Exo-PG (µg GalA·mg prot^{-1}·h^{-1})	70.70	92.80	77.70	102.80	9.37
Endo-PG (Δ viscosity in sec·mg prot^{-1}·h^{-1})	3.43	4.00	3.68	5.00	0.72
PE (mM NaOH·mg prot^{-1}·h^{-1})	0.17	0.34	0.26	0.27	0.01
L*	59.86	56.53	56.43	55.95	0.90
a*	-4.99	-10.01	-10.56	-10.36	0.47
b*	40.62	36.49	36.33	36.55	1.02
Chroma	41.39	37.85	37.79	38.00	1.00
Hue°	97.73	105.41	105.95	105.86	0.67
Visual colour (1-5)	1.73	1.02	1.01	1.02	0.15
Chlorophyll a (µg·g-1 FW skin)	75.90	107.00	106.70	111.60	8.83
Chlorophyll b (µg·g^{-1} FW skin)	29.15	43.20	40.98	45.20	5.44
Total chlorophylls (µg·g^{-1} FW skin)	105.00	150.10	147.70	156.80	13.21
Skin carotenoids (µg·g^{-1} FW)	34.70	30.70	30.64	31.18	ns
Pulp carotenoids (µg·g^{-1} FW)	21.34	7.48	8.84	7.33	0.90
Sucrose (µg·mg^{-1} FW)	42.09	37.05	31.33	34.23	2.97
Glucose (µg·mg^{-1} FW)	3.47	6.51	6.58	6.34	0.57
Fructose (µg·mg^{-1} FW)	23.52	27.30	23.54	24.22	1.28
Citric acid (µg·mg^{-1} FW)	5.93	7.15	7.88	7.53	0.64
Malic acid (µg·mg^{-1} FW)	3.48	3.81	3.91	3.90	ns
Succinic acid (µg·mg^{-1} FW)	77.02	92.42	86.40	90.45	4.39
Ascorbic acid (mg·100g^{-1} FW)	24.33	26.01	25.41	25.98	ns
Total antioxidants (µmol TE·100 g^{-1} FW)	3.83	4.056	3.43	3.54	0.14

ns = not significant at $P \leq 0.05$. NA = normal atmosphere, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2. Means (n = 150) are the average of 5 batches of of fruit assessed at removal after 0, 1, 2, 3 and 4 weeks of storage.

Table 4.2. Experiment 1: Mean firmness, activities of *exo*-PG, *endo*-PG , and PE enzymes, L*, a*, b*, chroma, hue°, visual colour, chlorophylls (a, b, and total), carotenoids (skin and pulp), sugars, organic acids, ascorbic acid, and total antioxidant in mango fruit influenced by storage period at 13°C

Parameters	Storage period (week)					LSD ($P \leq 0.05$)
	0	1	2	3	4	
Firmness (N)	127.26	124.46	99.47	53.23	37.68	4.02
Exo-PG (µg GalA·mg prot^{-1}·h^{-1})	71.10	85.20	63.40	53.50	56.80	10.88
Endo-PG (Δ viscosity in sec·mg prot^{-1}·h^{-1})	3.99	4.41	1.79	1.56	8.40	0.80
PE (mM NaOH·mg prot^{-1}·h^{-1})	0.42	0.43	0.31	0.07	0.08	0.01
L*	56.36	55.79	57.72	57.55	58.54	1.00
a*	-10.05	-10.77	-10.91	-10.21	-9.89	0.52
b*	36.46	36.59	37.81	37.83	38.81	1.13
Chroma	37.84	38.10	39.09	38.90	39.87	ns
Hue°	105.49	106.20	104.42	101.65	100.94	0.74
Visual colour (1-5)	1.00	1.01	1.18	1.30	1.48	0.17
Chlorophyll a (µg·g^{-1} FW skin)	120.20	94.50	102.80	96.80	87.20	9.87
Chlorophyll b (µg·g^{-1} FW skin)	46.76	38.18	40.29	33.85	39.10	ns
Total chlorophylls (µg·g^{-1} FW skin)	166.90	132.60	143.10	130.60	26.20	14.77
Skin carotenoids (µg·g^{-1} FW)	29.52	20.65	30.34	35.34	43.20	3.03
Pulp carotenoids (µg·g^{-1} FW)	7.12	6.00	6.57	16.92	19.23	1.00
Sucrose (µg·mg^{-1} FW)	17.77	24.02	36.29	59.80	42.98	2.97
Glucose (µg·mg^{-1} FW)	0	5.35	7.29	7.98	8.03	0.64
Fructose (µg·mg^{-1} FW)	24.92	21.08	22.90	29.78	24.54	1.43
Citric acid (µg·mg^{-1} FW)	3.37	8.12	10.42	7.59	6.13	0.72
Malic acid (µg·mg^{-1} FW)	4.03	4.00	3.94	3.73	3.18	0.29
Succinic acid (µg·mg^{-1} FW)	88.33	83.27	92.99	88.75	79.52	4.90
Ascorbic acid (mg·100g^{-1} FW)	26.54	38.17	18.61	16.43	27.42	1.70
Total antioxidant (µmol TE·100 g^{-1} FW)	4.24	4.16	4.20	3.41	2.56	0.15

ns = not significant at $P \leq 0.05$. Means (n = 120) are the average of 4 batches of of fruit from 4 treatments in either air or under an atmosphere of 3% O_2 combined with either 4, 5, or 6% CO_2.

4.3.2. *Experiment 2:* The influence of controlled atmosphere storage on ripening of 'Kensington Pride' mango

The mean values of parameters measured during 4-day ripening period in 'Kensington pride' mango fruit are presented in Table 4.3 and Table 4.4. Storage conditions (i.e. the average of 5 ripening times) affected most parameters except firmness, *endo*-PG, the contents of sucrose and malic acid, and total antioxidants. Likewise, ripening time (i.e. the average of all 4 treatments) affected most parameters except the content of sucrose, glucose, citric, malic and ascorbic acid, and total antioxidants.

4.3.2.1. Fruit firmness

Ripening time significantly ($P \leq 0.05$) affected fruit firmness irrespective of storage atmosphere (Table 4.4), whereas storage condition based on the average of the 5 ripening times had no significant effect on fruit firmness (Table 4.3). The interaction between storage atmospheres and ripening time did not significantly ($P \leq 0.05$) influence fruit firmness (Figure 4.11). During ripening, the firmness of fruit from NA storage decreased by 25% within 2 days. The fruit stored in CA1 and CA2 started to loss their firmness after one day ripening period (30.12% and 23.30%, respectively). However, fruit stored in NA were not significantly firmer than those stored in CA when ripe at day 4, and no significant changes were noticed in CA3-stored fruit during ripening.

4.3.2.2.Fruit softening enzyme activities

4.3.2.2.1. Exo- and endo-polygalacturonase enzyme activities

Different storage atmospheres and ripening time significantly ($P \leq 0.05$) affected the activity of *exo*-PG enzyme (Table 4.3 and 4.4). The interaction between storage atmospheres and ripening time significantly ($P \leq 0.05$) influenced the activity of *exo*-PG enzyme (Figure 4.12). NA-stored fruit exhibited significant increase in *ex*o-PG activity after 3 days ripening (5-fold), but this enzyme was significantly active after 2 days ripening (3-fold) in CA1 fruit, and 1 day ripening i.e., 4-fold and 4.8-fold in CA2 and CA3 fruit, respectively. At removal from storage (i.e. ripening time 0), CA1 and CA3- stored fruit had the highest *exo*-PG activity (40.41 and 38.81 μg GalA released·mg $prot^{-1}·h^{-1}$, respectively), followed by CA2-stored fruit (32.96 μg GalA released·mg $prot^{-1}·h^{-1}$), and then NA-stored fruit (28.64 μg GalA released·mg

prot^{-1}·h^{-1}). At the end of ripening time (day 4), higher activity remained in CA-stored fruit (108.56, 106.00, and 97.75 μg GalA released·mg prot^{-1}·h^{-1} in CA2, CA3, and CA1, respectively) compared to that in NA-stored fruit (86.45 μg GalA released·mg prot^{-1}·h^{-1}).

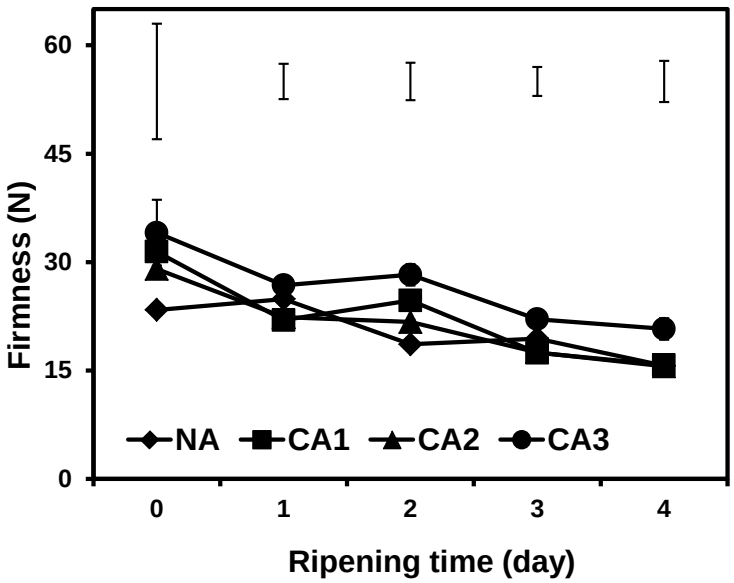

Figure 4.11. Effects of storage atmospheres (SA) and ripening time (RT) on firmness of mango fruit during ripening. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 15, 3 replications with 5 fruit in each replication). LSD ($P \leq 0.05$): SA = ns, RT = 3.69, SA x RT = ns (7.38). NA = normal atmosphere, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2, ns = not significant.

Ripening time, regardless of storage atmospheres, significantly ($P \leq 0.05$) affected *endo*-PG activity. The interaction between storage atmospheres and ripening time also significantly ($P \leq 0.05$) affected *endo*-PG activity (Figure 4.12). The activity of *endo*-PG in NA-stored fruit were undetected at the end of storage to the first day of ripening but increased to its peak (viscosity changes in 3.55 sec·mg prot^{-1}·h^{-1}) on day 3 in ripening. CA1 reached the maximum *endo*-PG activity also at day 3 but CA2 and CA3 at day 2 during ripening period. The highest activity *endo*-PG exhibited in NA- and CA1-stored fruit (viscosity changes in 2.86 and 2.74 sec·mg prot^{-1}·h^{-1}, respectively), followed by CA2-stored fruit (viscosity changes in 1.710 sec·mg prot^{-1}·h^{-1}), and CA3-stored fruit (viscosity changes in 0.09 sec·mg prot^{-1}·h^{-1}) at the last day of ripening.

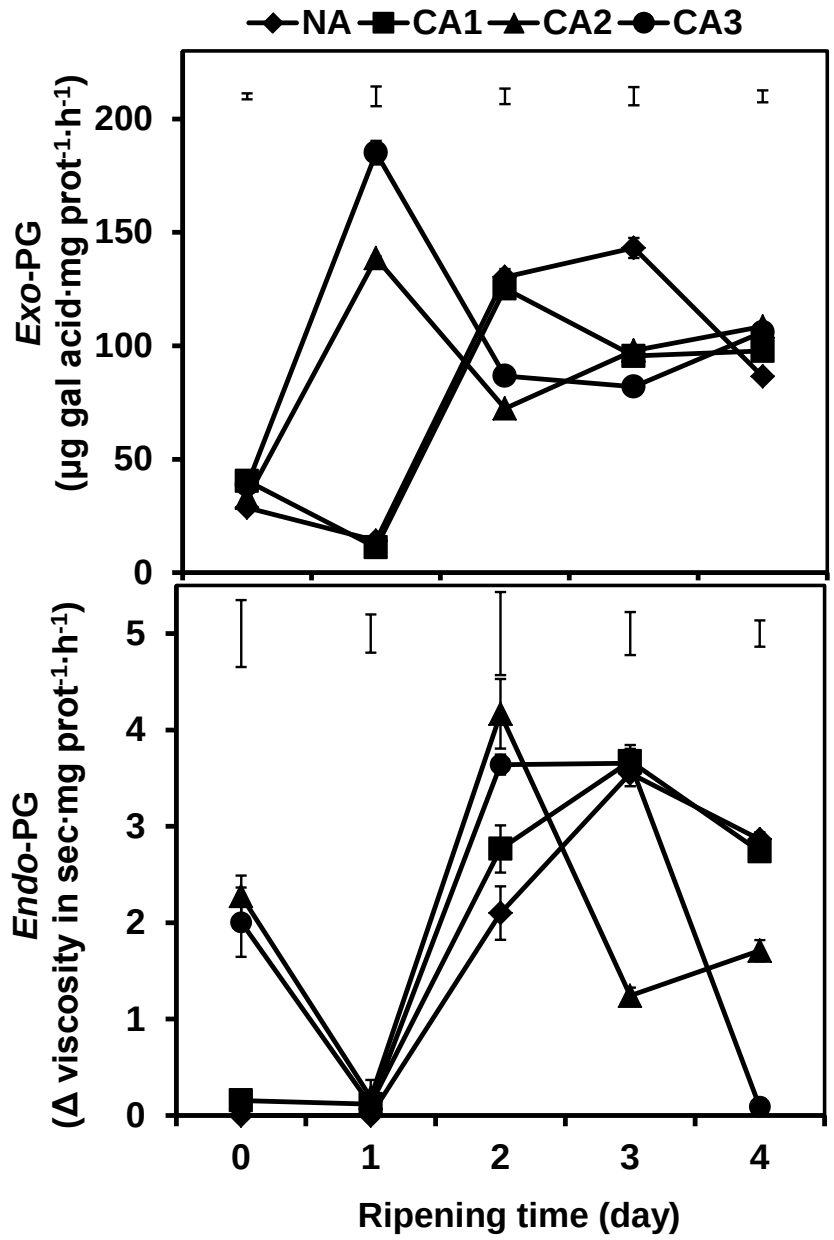

Figure 4.12. *Exo*- and *endo*-polygalacturonase activities in fruit pulp during ripening time (RT) after storage at different atmospheres (SA). Vertical bars out side graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$) for *exo*-PG: SA = 2.61, RT = 2.92, SA x RT = 5.84; *endo*-PG: SA = ns, RT = 0.25, SA x RT = 0.50. NA = normal atmosphere, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2, ns = not significant.

4.3.2.2.2. Pectin esterase enzyme activity

Different storage atmospheres and ripening time significantly ($P \leq 0.05$) influenced the activity of PE enzyme (Figure 4.13.). The highest activity of PE enzyme detected after storage was in CA2-stored fruit at the first day of ripening (67.05 μM NaOH·mg $prot^{-1}·h^{-1}$), which was almost doubled from other CA-stored fruit and about 1.5 times from NA-stored fruit PE enzyme activity increased significantly in NA-stored fruit at the second day of ripening, then it decreased onwards. Similarly, PE activity in CA1-stored fruit significantly increased and reached its maximum at the second day, declined to its minimum activity on the third day and remained steady until the end of ripening time. This phenomenon was also exhibited in CA2-stored fruit. On the other hand, CA3-stored fruit exhibited maximum PE enzyme activity at the first day, remained unchanged and continuously decreased until the fruit were fully ripe on day 4. The interaction between storage atmosphere and ripening time significantly ($P \leq 0.05$) affected the PE activity.

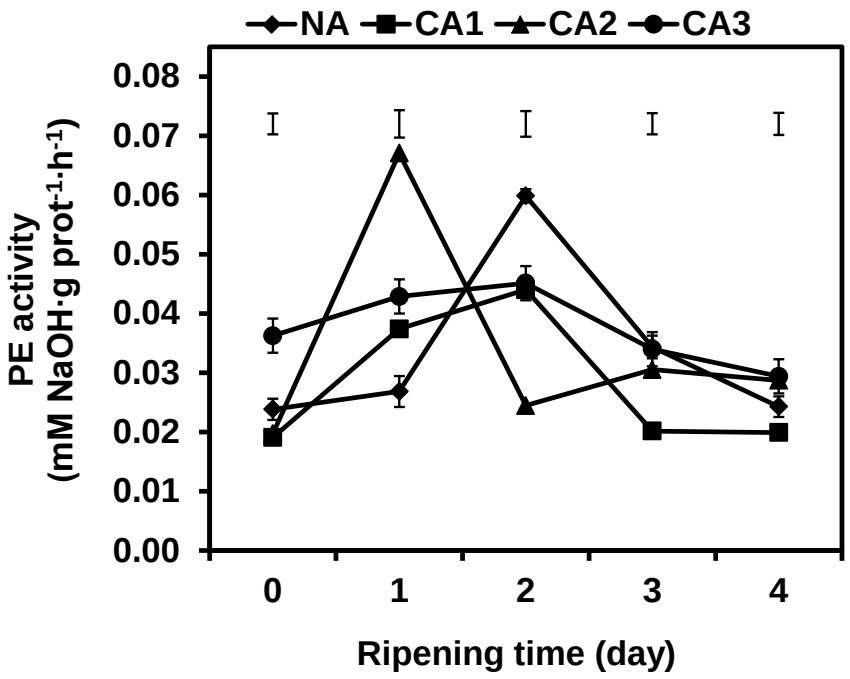

Figure 4.13. Pectin esterase (PE) activity in fruit pulp during ripening time (RT) after storage at different atmosphere storage compositions (SA). Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$): SA = 0.002, RT = 0.002, SA x RT = 0.004. NA = normal atmosphere, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2.

4.3.2.3.Fruit colour

Fruit colour lightness (L*), a*, b*, and colour saturation (C*) were significantly ($P \leq 0.05$) higher in NA-stored fruit than in CA-stored fruit started from day 0 throughout the ripening period (Figure 4.14). More yellow colour development was also supported by lower hue angle value exhibited in NA-stored fruit than in CA-stored fruit. During ripening, no significant colour development were noticed in L*, a*, b, C*, and hue angle of CA3-stored fruit. Visually, fruit stored under NA and CA conditions showed highest yellow colour development at day 4 in ripening although it was not significantly ($P \leq 0.05$) between CA1- and CA2-stored fruit, and CA2- and CA3-stored fruit. No significant ($P \leq 0.05$) interaction between storage atmospheres and storage time was noticed through spectrophotometer but visual observation.

4.3.2.4.Fruit skin pigment

The levels of chlorophylls and carotenoids in fruit skin were significantly ($P \leq 0.05$) affected with storage atmosphere and ripening time. NA-stored fruit exhibited the highest decrease in total chlorophylls (about 45%) at first day of ripening whereas 24% loss was observed in CA3-stored fruit at the same day. CA1- and CA2-stored fruit showed decreasing chlorophylls levels (24% and 26%, respectively) after second day of ripening. Further decrease in the total chlorophylls level was recorded in CA2-stored fruit at third day of ripening but fruit from other storage treatments showed no significant decrease. Comparing the loss of total chlorphylls from the beginning towards the end of ripening period, CA3-stored fruit showed the highest reduction (127.15 $\mu g \cdot g^{-1}$ FW skin), followed by NA-stored fruit (111.65 $\mu g \cdot g^{-1}$ FW), CA2- stored fruit (100.84 $\mu g \cdot g^{-1}$ FW), then CA1-stored fruit (71.82 $\mu g \cdot g^{-1}$ FW). In spite of its highest reduction in total chlorophylls levels, CA3-stored fruit demonstrated the highest total chlorophylls level compared to other storage atmosphere treated fruit during entire ripening time (Figure 4.15).

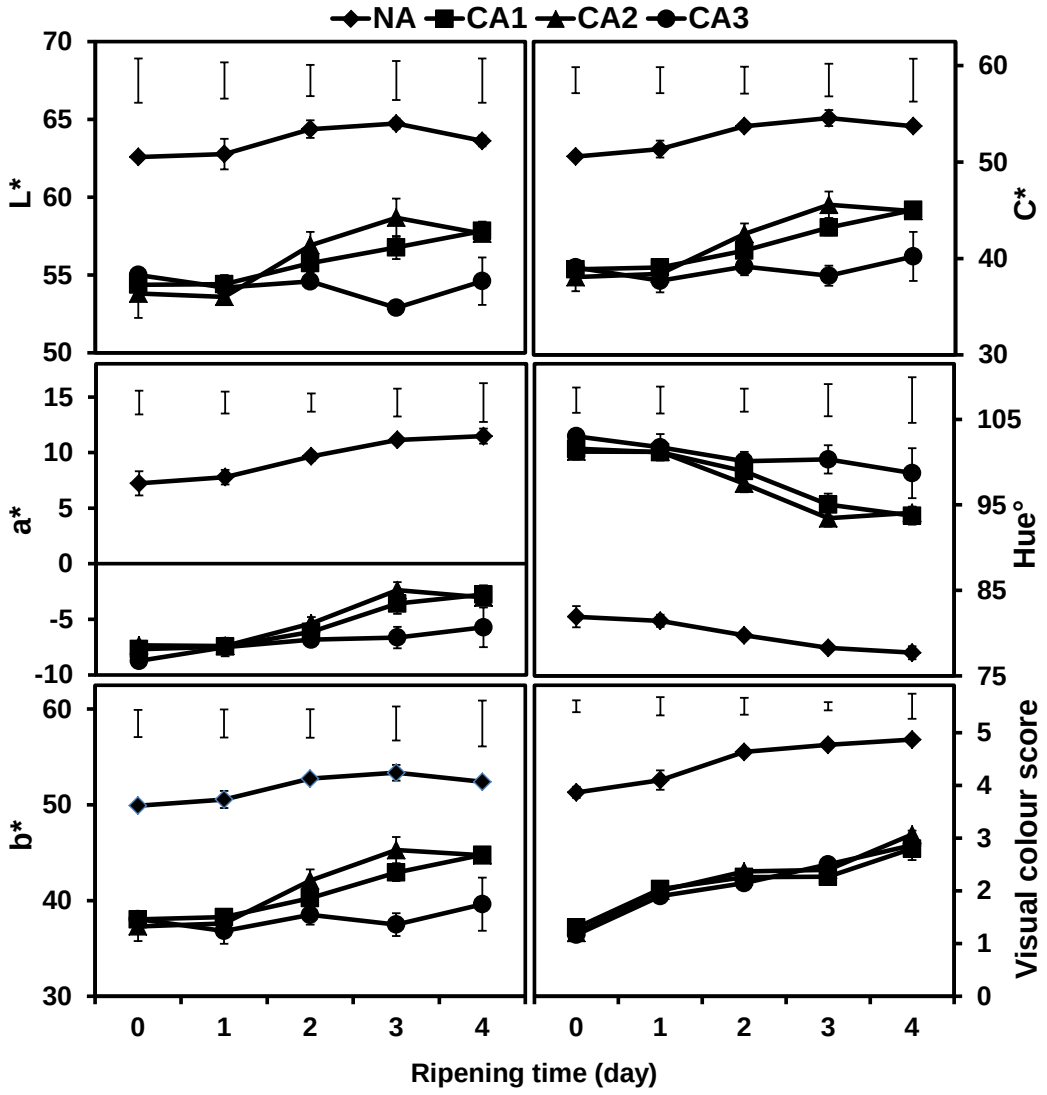

Figure 4.14. Effects of storage atmospheres (SA) and ripening time (RT) on fruit skin colour during ripening. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 15, 3 replications with 5 fruit in each replication). LSD ($P \leq 0.05$) for L*: SA = 0.99, RT = 1.11, SA x RT = ns; a*: SA= 0.95, RT = 1.06, SA x RT = ns; b*: SA = 1.37, RT = 1.53, SA x RT = ns; chroma: SA = 1.28, RT = 1.43, SA x RT = ns; Hue°: SA = 1.45, RT = 1.62, SA x RT = ns; visual colour: SA = 0.13, RT = 0.14, SA x RT = 0.28. NA = normal atmosphere, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2, ns = not significant.

Significant declining amount of skin carotenoids was observed in NA-stored fruit during four days ripening period but not in CA-stored fruit. CA1- and CA3-stored fruit showed significantly decreased carotenoids levels at the first day of ripening and remained constant throughout the rest of ripening period. Carotenoids in

all CA-stored fruit showed significantly higher carotenoids levels (2.97, 3.06, and 2.92 µg·g^{-1} FW in CA1-, CA2-, and CA3-stored fruit, respectively) than NA-stored fruit (0.23 µg·g^{-1} FW) at day 4, although no significant different between them at the beginning and at day 2 and 3 of ripening time (Figure 4.15). Interaction between storage atmosphere and ripening time did not significantly ($P \leq 0.05$) affect fruit skin pigments.

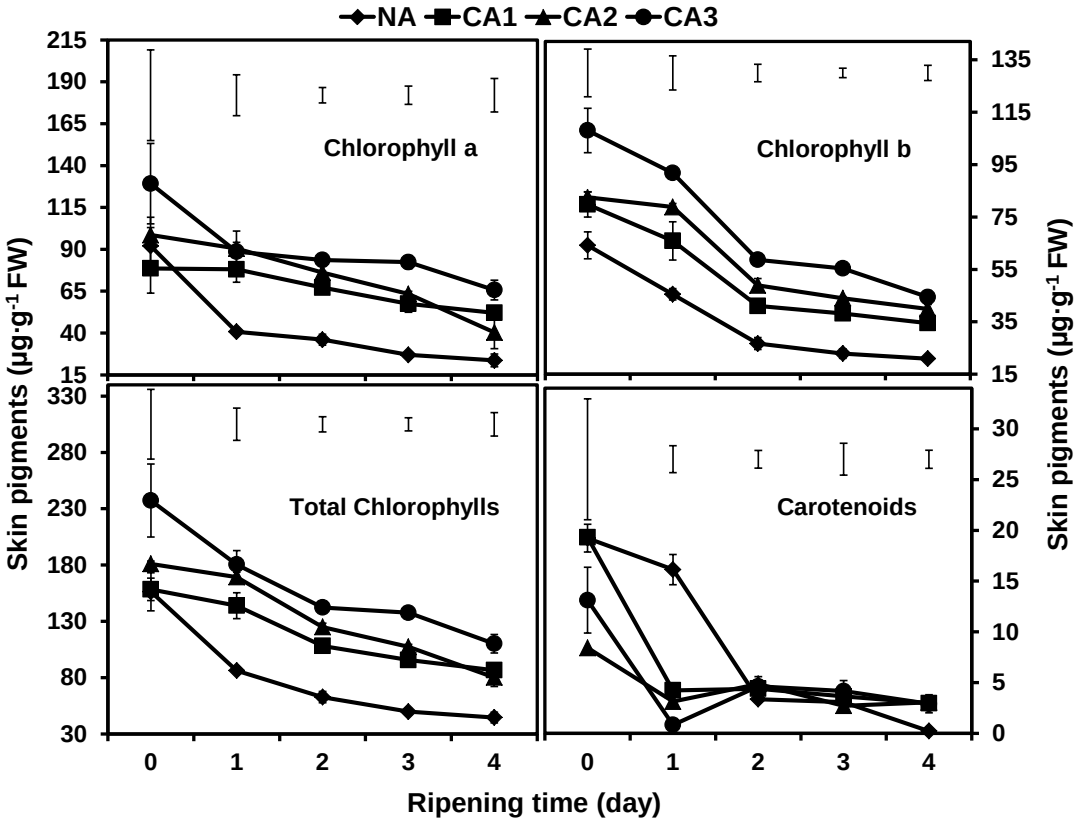

Figure 4.15. Effects of storage atmospheres (SA) and ripening time (RT) on skin chlorophyll and carotenoid levels in mango fruit during ripening.. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$): Chlorophyll a: SA = 9.81, RT =10.97, SA x RT = ns; Chlorophyll b: SA = 3.77, RT = 4.22, SA x RT = ns; total chlorophylls: SA = 13.09, RT = 14.64, SA x RT = ns; carotenoids: SA = 2.32, RT = 2.59, SA x RT = ns. NA = normal atmosphere, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2, ns = not significant.

4.3.2.5.Sugars and organic acids

4.3.2.5.1. Sugars

The sucrose level in mango pulp was not significantly ($P \leq 0.05$) affected by storage atmosphere and ripening time (Table 4.3 and Table 4.4). However, sucrose level reduced significantly at the end of ripening period in all storage treatments. CA1-stored fruit lost about 29%, followed by NA-stored fruit (28%), CA3-stored fruit (26%), and the lowest loss was exhibited in CA2-stored fruit (16%) of its sucrose level when fully ripe at day 4. There were no significant differences in sucrose content between NA-stored fruit and CA2-stored fruit, and among CA-stored fruit at ripe stage (Figure 4.16).

Different storage atmospheres significantly ($P \leq 0.05$) affected glucose level. However, ripening time did not significantly affect its level (Figure 4.16). Glucose in NA-stored fruit was not detected after 4 weeks storage and following 3 days in ripening period which finally increased significantly at day 4. CA1- and CA2-stored fruit exhibited non-significant changes during ripening time, whilst CA3-stored fruit showed significant change at day 3 and remained stable to day 4 of ripening period. Comparing the glucose levels in different storage atmospheres treated fruit at day 4, no significant differences among NA-stored, CA1-, and CA2-stored fruit; and among all CA-stored fruit.

Storage atmospheres and ripening time significantly ($P \leq 0.05$) affected fructose level in KP mango fruit. Fruit from NA, CA1 and CA3 storage did not show significant difference in fructose levels at the end of storage (Figure 4.16). NA-stored and CA2 stored fruit also showed no significant difference in their fructose level before ripening period. The fruit stored in different atmospheres did not exhibit significant difference of fructose content at day 1 to day 3 during ripening. However, CA3-stored fruit showed significantly lower fructose content (34.65 $\mu g \cdot mg^{-1}$ FW pulp) than NA-, CA1-, and CA2-stored fruit (38.75, 39.69, and 42.30 $\mu g \cdot mg^{-1}$ FW pulp, respectively). The interaction between storage atmosphere and ripening time did not significantly ($P \leq 0.05$) affect fructose level in mango fruit pulp.

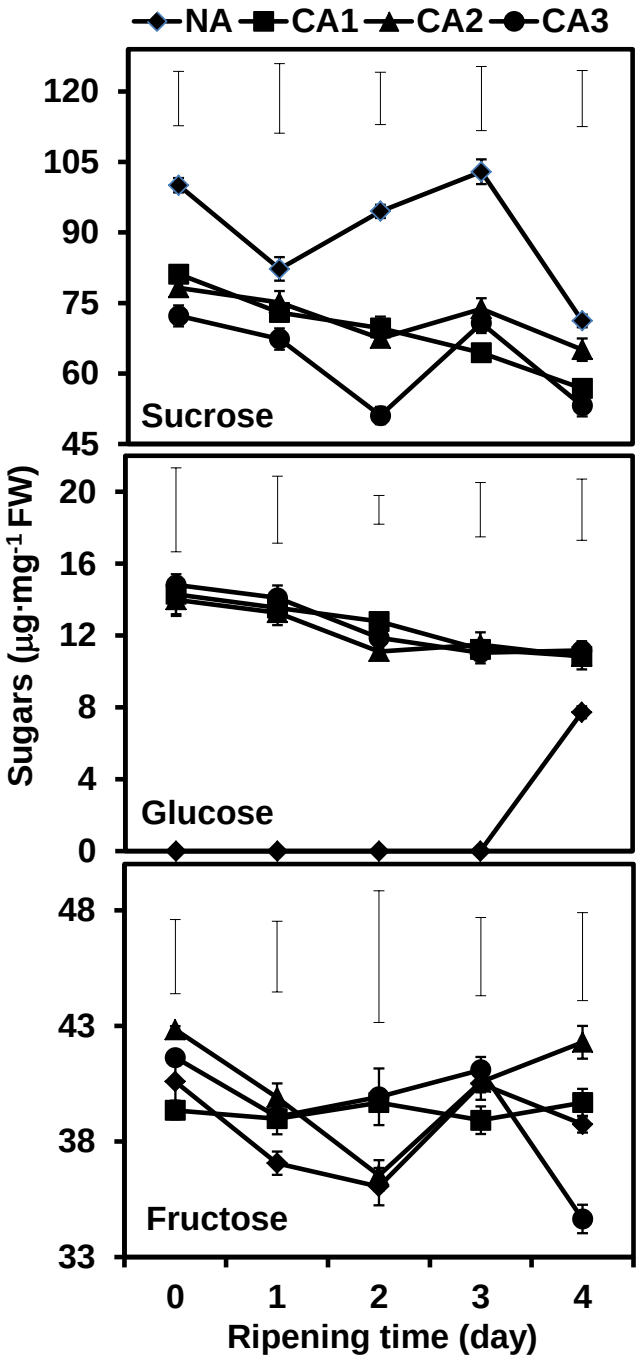

Figure 4.16. Effects of storage atmosphere (SA) and ripening time (RT) on levels of different sugars in mango fruit pulp during ripening. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$) for sucrose: SA = ns, RT = ns, SA x RT = ns; glucose: SA= 1.35, RT = ns, SA* RT = 3.01; fructose: SA = 4.97, RT = 5.56, SA x RT = ns. NA = normal atmosphere, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2, ns = not significant.

4.3.2.5.2. Organic acids

Storage atmospheres and ripening time significantly ($P \leq 0.05$) affected citric and succinic acids levels in fruit pulp (Figure 4.17). Citric acid level in NA- and all CA-stored fruit decreased significantly as the ripening started at the first day. The level of citric acid declined continuously in NA-stored fruit until the third day of ripening and remained unchanged to day 4. CA1-stored fruit also showed significant decrease in citric acid level on day 2, remained stable on day 3 before decreasing again on day 4. CA2- and CA3-stored fruit did not show significant loss in citric acid levels after second day but on the fourth day of ripening period. More than 50% loss was noticed in NA-, CA1- and CA2-stored fruit, and only 37% was noticed in CA3-stored fruit at the end of the ripening time (Figure 4.17). At the ripe stage, the citric acid level in all CA-stored fruit was significantly higher than NA-stored fruit.

There were no significant effects of storage atmospheres and storage time on malic acid levels in mango fruit pulp (Table 4.3 and 4.4). However, the interaction between storage atmospheres and storage time significantly ($P \leq 0.05$) affected malic acid levels. The fruit stored in CA1 exhibited significant change in malic acid level at 4 days ripening whilst CA3-stored fruit started at day 2 and remained unchanged throughout ripening. At the end of ripening period, malic acid level in NA-stored fruit was 4.03 $\mu g \cdot mg^{-1}$ which was not significantly different from that in CA1-stored fruit (3.74 $\mu g \cdot mg^{-1}$). All CA-stored fruit exhibited similar amount of malic acid at the ripe stage (Figure 4.17).

All storage atmosphere treatments showed significant ($P \leq 0.05$) decrease in succinic acid level during ripening. CA3-stored fruit underwent the highest loss (52.7%), followed by NA-stored fruit (44%), CA1-stored fruit (39%), and then CA2-stored fruit (21%). The succinic acid level in fully ripe CA1- and CA3- stored fruit was not significantly different (Figure 4.17).

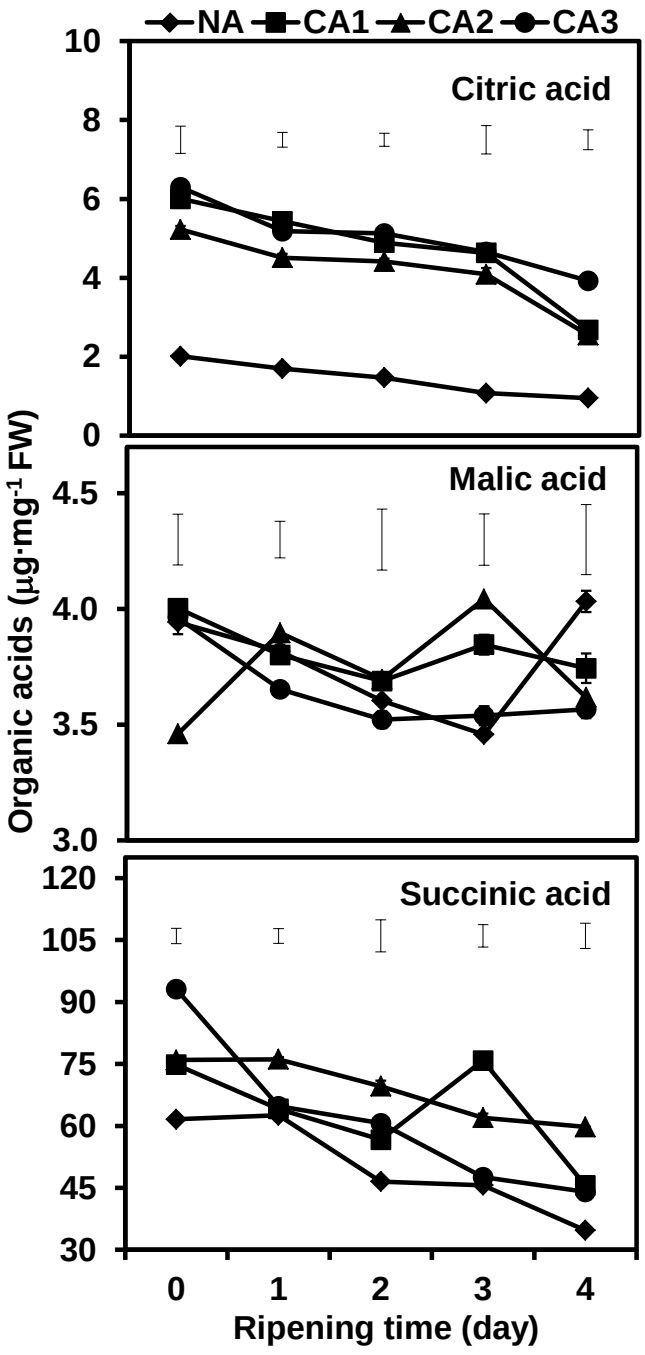

Figure 4.17. Effects of different storage atmospheres (SA) and ripening time (RT) on different organic acids in mango fruit pulp during ripening. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM, (n = 3 replications). LSD ($P \leq 0.05$) for citric acid: SA = 0.22, RT = 0.24, SA x RT = 0.48; malic acid: SA = ns, RT = ns, SA x RT = 0.21; succinic: SA = 2.17, RT = 2.43, SA x RT = 4.86. NA = normal atmosphere, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2, ns = not significant.

4.3.2.6.Carotenoids, ascorbic acid, and total antioxidants in mango pulp

Different storage atmospheres and storage time significantly ($P \leq 0.05$) affected carotenoids levels in mango pulp (Table 4.3 and 4.4). The interaction between storage atmospheres and storage time also significantly ($P \leq 0.05$) influenced carotenoids levels (Figure 4.18.). The fruit exhibited significantly increasing carotenoids levels at the first day of ripening. All fruit attained their highest carotenoids levels at day 4 although they exhibited significantly different amount. At the beginning of ripening stage, NA-stored fruit contained the highest amount of carotenoids (53.86 $\mu g \cdot g^{-1}$), followed by CA1 (22.24 $\mu g \cdot g^{-1}$), CA2 (18.47 $\mu g \cdot g^{-1}$), and then CA3-stored fruit (13.01 $\mu g \cdot g^{-1}$). However, all CA-stored fruit produced carotenoids more than 2 folds (2.2, 3.6, and 2.7 folds in CA1, CA2, and CA3 treated fruit, respectively) whilst NA-stored fruit only gained 1.6 folds at day 4 in ripening period compared to the fruit after storage (Figure 4.19.).

Storage atmospheres significantly ($P \leq 0.05$) influenced the ascorbic acid levels in KP mango pulp. Higher amount of ascorbic acid were recorded in all CA-stored fruit compared to NA-stored fruit during ripening. However, CA-stored fruit gained less than 1% of ascorbic acid whereas NA-stored fruit produced about 24% of this acid during ripening period (Figure 4.19). Across all atmosphere conditions, there was little effect of ripening time and on ascorbic acid content (Table 4.4).

Storage atmosphere, ripening time, and their interaction did not significantly ($P \leq 0.05$) affect total antioxidant activity in KP mango fruit pulp (Table 4.3 and 4.4). Total antioxidant activity in different storage treated fruit remained unchanged during ripening and showed no significant different among them at the end of ripening period (Figure 4.20).

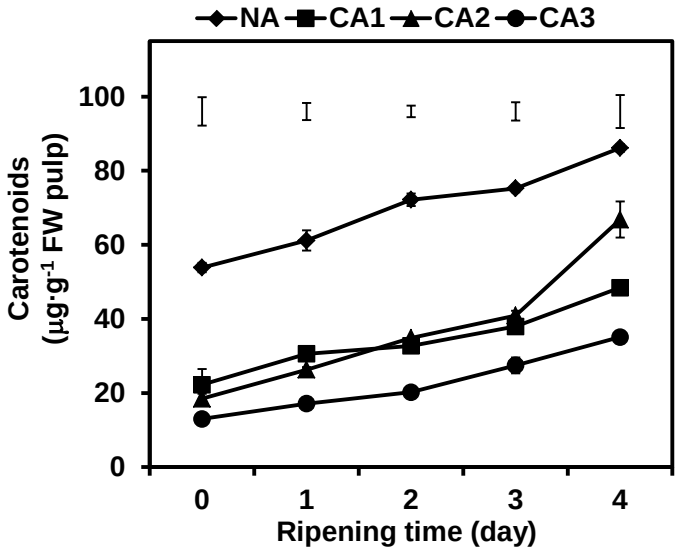

Figure 4.18. Effects of storage atmosphere (SA) and ripening time (RT) on carotenoids level in mango fruit pulp during ripening. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$): SA = 2.44, RT = 2.73, SA x RT = 5.46. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2.

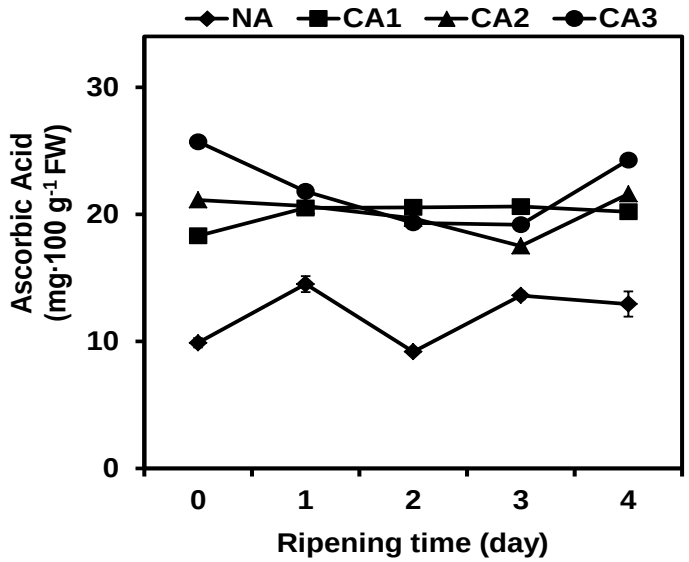

Figure 4.19. Effects of storage atmosphere (SA) and ripening time (RT) on ascorbic acid level in mango fruit pulp during ripening. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$): SA = 1.51, RT = ns, SA x RT = ns. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2, ns = not significant.

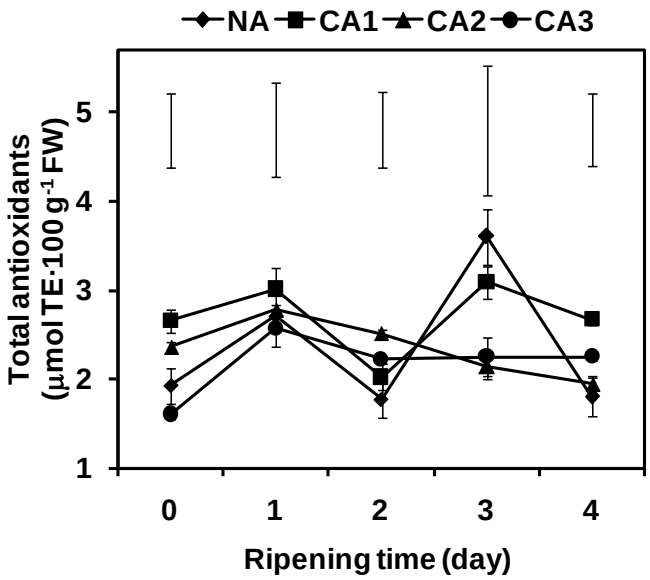

Figure 4.20. Effects of storage atmosphere (SA) and ripening time (RT) on the level of total antioxidants in mango fruit pulp during ripening. Vertical bars outside graphs represent LSD ($P \leq 0.05$), vertical bars within graphs represent SEM (n = 3 replications). LSD ($P \leq 0.05$): SA = ns, RT = ns, SA x RT = ns. NA = normal air, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2, ns = not significant.

Table 4.3. Experiment 2: Mean firmness, activities of *exo*-PG, *endo*-PG , and PE enzymes, L*, a*, b*, chroma, hue°, visual colour, chlorophylls (a, b, and total), carotenoids (skin and pulp), sugars, organic acids, ascorbic acid, and total antioxidants in mango fruit influenced by different atmosphere compositions after 4 weeks of storage at 13°C

Parameters	Storage atmospheres				LSD
	NA	CA1	CA2	CA3	($P \leq 0.05$)
Firmness (N)	20.40	22.26	21.25	26.40	ns
Exo-PG (µg GalA·mg prot^{-1}·h^{-1})	80.54	74.02	90.09	99.73	2.61
Endo-PG (Δ viscosity in sec·mg prot^{-1}·h^{-1})	1.70	1.89	1.92	1.90	ns
PE (mM NaOH·mg prot^{-1}·h^{-1})	0.03	0.03	0.03	0.04	0.002
L*	63.62	55.83	56.13	54.25	0.99
a*	9.46	-5.53	-5.10	-7.09	0.95
b*	51.79	40.85	41.40	38.10	1.37
Chroma	52.79	41.40	41.90	38.87	1.28
Hue°	79.83	98.08	97.48	100.78	1.45
Visual colour (1-5)	4.45	2.13	2.76	2.65	0.13
Chlorophyll a (µg·g-1 FW skin)	43.87	66.64	73.74	89.90	9.81
Chlorophyll b (µg·g^{-1} FW skin)	36.04	51.88	58.82	71.66	3.77
Total chlorophylls (µg·g^{-1} FW skin)	79.91	118.53	132.55	161.55	13.09
Skin carotenoids (µg·g^{-1} FW skin)	8.41	6.92	4.44	5.14	2.32
Pulp carotenoids (µg·g^{-1} FW)	69.71	34.39	37.47	22.58	2.44
Sucrose (µg·mg^{-1} FW)	90.17	69.01	71.92	62.90	ns
Glucose (µg·mg^{-1} FW)	1.55	12.54	12.14	12.60	1.35
Fructose (µg·mg^{-1} FW)	38.59	39.33	40.43	39.28	ns
Citric acid (µg·mg^{-1} FW)	1.44	4.73	4.16	5.04	0.22
Malic acid (µg·mg^{-1} FW)	3.77	3.82	3.74	3.65	ns
Succinic acid (µg·mg^{-1} FW)	50.22	63.39	68.68	62.03	2.17
Ascorbic acid (mg·100g^{-1} FW)	12.02	20.03	20.12	22.06	1.51
Total antioxidants (µmol TE·100 g^{-1} FW)	2.36	2.69	2.34	2.18	ns

ns = not significant at $P \leq 0.05$. NA = normal atmosphere, CA1 = 3% O_2 + 4% CO_2, CA2 = 3% O_2 + 5% CO_2, CA3 = 3% O_2 + 6% CO_2. Means (n = 150) are the average of 5 batches of fruit assessed at 0, 1, 2, 3, and 4 days of ripening at 21°C.

Table 4.4. Experiment 2: Mean firmness, activities of *exo*-PG, *endo*-PG , and PE enzymes, L*, a*, b*, chroma, hue°, visual colour, chlorophylls (a, b, and total), carotenoids (skin and pulp), sugars, organic acids, ascorbic acid, and total antioxidant in mango fruit influenced by ripening time

Parameters	Ripening time (day)					LSD
	0	1	2	3	4	(P ≤ 0.05)
Firmness (N)	29.49	24.01	23.32	19.12	16.94	3.69
Exo-PG (μg GalA·mg prot^{-1}·h^{-1})	35.21	87.25	103.67	104.65	99.69	2.92
Endo-PG (Δ viscosity in sec·mg prot^{-1}·h^{-1})	1.11	0.10	3.17	3.03	1.85	0.25
PE (mM NaOH·mg prot^{-1}·h^{-1})	0.02	0.04	0.04	0.03	0.03	0.002
L*	56.44	56.24	57.91	58.27	58.43	1.11
a*	-4.14	-3.64	-2.17	-0.37	-0.01	1.06
b*	40.83	40.82	43.40	44.76	45.38	1.53
Chroma	41.65	41.62	44.06	45.39	45.97	1.43
Hue°	96.94	96.38	94.06	91.77	91.06	1.62
Visual colour (1-5)	1.88	2.51	2.85	2.98	3.40	0.14
Chlorophyll a (μg·g^{-1} FW skin)	99.60	74.46	65.70	57.49	45.43	10.97
Chlorophyll b (μg·g^{-1} FW skin)	89.62	70.50	43.83	40.10	34.93	4.22
Total chlorophylls (μg·g^{-1} FW skin)	183.22	144.96	109.53	97.60	80.36	14.64
Skin carotenoids (μg·g^{-1} FW skin)	15.02	6.09	4.32	3.41	2.30	2.59
Pulp carotenoids (μg·g^{-1} FW)	26.89	33.80	40.00	45.38	59.12	2.73
Sucrose (μg·mg^{-1} FW)	82.91	74.41	70.67	77.96	61.56	ns
Glucose (μg·mg^{-1} FW)	10.78	10.23	8.94	8.44	10.15	ns
Fructose (μg·mg^{-1} FW)	41.40	38.76	38.05	40.28	38.85	ns
Citric acid (μg·mg^{-1} FW)	4.88	4.21	3.97	3.61	2.53	ns
Malic acid (μg·mg^{-1} FW)	3.84	3.79	3.63	3.72	3.74	ns
Succinic acid (μg·mg^{-1} FW)	76.37	66.89	58.36	57.75	46.02	2.43
Ascorbic acid (mg·100g^{-1} FW)	18.76	19.37	17.18	17.72	19.75	ns
Total antioxidant (μmol TE·100 g^{-1} FW)	2.13	2.76	2.13	2.77	2.16	ns

ns = not significant at $P \leq 0.05$. Means (n = 120) are the average of 4 batches of fruit from 4 treatments after 4 weeks of storage at 13°C in either air or under an atmosphere of 3% O_2 combined with either 4, 5, or 6% CO_2

4.4. Discussion

4.4.1. Fruit softening

Reduced O_2 (3%) and elevated CO_2 (4, 5, and 6%) in CA storage was more effective in maintaining fruit firmness compared to NA storage (Table 4.1). Fruit stored in CA containing 3% O_2 and 5% CO_2 were firmer than those stored in other atmospheres after 4 weeks (Figure 4.1). The higher fruit firmness in CA-stored fruit may be attributed to the retardation of fruit ripening process due to the low O_2 and high CO_2 levels in storage atmospheres (Batu and Thompson, 1998; Lalel et al., 2003a, 2005) as the fruit softening is associated with the advancement of ripening in mango (Noomhorm and Tiasuwan, 1995). CA storage has been previously demonstrated to maintain the fruit firmness during storage of mango (Abdulah and Basiouny, 2000; Kader, 1993; Lalel et al., 2003a, 2005; McLauchlan and Barker, 1992; Noomhorm and Tiasuwan, 1995). It may also be argued that the maintenance of fruit firmness during CA storage is due to the inhibition of fruit softening enzymes, specifically PE enzyme activity (Figure 4.3). CA retards the breakdown of pectin substances thus retarding the fruit softening process (Wills et al., 1998) which related to pectolytic enzymes activity (Kays and Paull, 2004).

Fruit from CA storage comprising of 3% O_2 and 6% CO_2 maintained its firmness better than fruit from other CA and NA conditions until day 4 during ripening. Although higher rate of fruit softening occurred during ripening in all CA-stored fruit as compared to the NA-stored, no significant differences in ripe fruit firmness were noticed irrespective of the storage atmospheres. My results support those reported by Yahia and Hernandez (1993) that the fruit firmness reduced significantly during ripening of CA-stored fruit. This high rate of fruit softening (Figure 4.11) may be due to high degradation of pectin substances as the result of high pectolytic enzymes activities after CA storage (Figure 4.12 and 4.13) when the fruit gained their normal metabolism process (Mathooko, 1996b). The PE hydrolyses the methyl ester chain resulting in free carboxyl group which is readily available for further degradation by PG enzymes (Kays and Paull, 2004). In normal ripening process, the cell walls of fruit suffer a natural degradation hence weaken the cell wall firmness and intercellular adhesion (Toivonen and Brummell, 2008).

In general, all CA conditions in this experiment delayed the activity of *exo*- and *endo*-PG up to 3 weeks storage (Figure 4.2). The activity of PE enzyme in fruit stored under CA and NA conditions decreased continuously during storage; although slower rate of reduction was monitored during storage in 5% CO_2 (Figure 4.3). These results suggested that CA storage was more effective than NA storage to delay the activity of pectolytic enzymes hence maintained better fruit firmness during storage (Lalel et al., 2001).

During ripening, all the pectolytic enzymes in fruit stored in 5% and 6% CO_2 activated earlier than those treated in NA. The activity of PE during ripening of fruit stored under those conditions increased at day 1 (Figure 4.13), suggested that high CO_2 storage enhanced the PE activity at the initial stage of ripening. Yashoda et al. (2007) also reported that enhanced activity of PE enzyme in the initial stage of mango ripening is necessary in order to produce demethylated pectin which in turn prepared for the subsequent action of PG enzyme. In unripe mango fruit, PG activity is barely detected (Yashoda et al., 2007) however in fully ripe fruit, which were treated in 5% and 6% CO_2 storage, exhibited high *exo*-PG and PE activity but low *endo*-PG activity (Figure 4.12 and 4.13). This occurrence suggested that the action of PG enzymes under the influence of CA storage was more active on the terminal chain than the inner part of demethylated pectin. In previous study, Chaimanee et al. (2000) reported that *exo*-PG activities in mature green 'Nam Dok Mai' mango increased as the ripening progressed, but *endo*-PG remained constant.

4.4.2. Fruit colour and pigments

CA storage was more effective in retarding the fruit skin colour changes during 4 weeks of storage at 13°C compared to those stored in NA (Figure 4.4). Irrespective of the atmosphere composition, CA-stored fruit exhibited lower chromaticity values, L*, a*, b*, chroma, and visual colour score and higher hue angle as compared to the NA-stored fruit. The residual effect of CA on limiting the skin colour development was also recorded during ripening of all CA-stored fruit. Similar effects of CA storage in retarding skin colour changes in mango have been reported earlier (Gonzalez-Aguilar et al., 1997; Lalel et al., 2005; McLauchlan and Barker, 1992; Noomhorm and Tiasuwan, 1995).

Chlorophylls degradation was recorded both in CA- and NA-stored fruit during storage and ripening although it was less pronounced in CA-stored fruit than in fruit kept under NA (Figure 4.5 and 4.15). Contrarily, the carotenoids levels were increased during storage, but decreased during ripening. These incidences indicated that the ripening process of the fruit started during storage and continued to ripening under normal air. High CO_2 (5% or 6% CO_2) in CA storage reduced the degradation of chlorophylls and maintained the carotenoids levels better then other atmosphere conditions during storage and ripening (Figure 4.5 and 4.15). Storage under normal air at 20°C resulting in decreasing chlorophylls has been reported by Wang et al. (2008a) in contrast to the effects of low O_2 and/or high CO_2 storage reducing the breakdown of chlorophylls (Bender et al., 2000c; Lalel et al., 2005; Rao and Rao, 2008; Thompson, 1996). Reducing chlorophylls and carotenoids during ripening of CA stored fruit may be due to senescence process (Jha et al., 2006; Kays, 1991; Kays and Paull, 2004; Mitra and Baldwin, 1997; Thomas, 1975) that proceeds under normal atmosphere and temperature.

The fruit skin colour is the reflection of pigments (Kays and Paull, 2004). The reduced carotenoids synthesis is reflected in a paler skin colour (Chaplin et al., 1991) and lower chroma value (Brecht et al., 2003). Carotenoids synthesis as reflected in yellow colour development was almost unnoticed visually during CA storage (Figure 4.4 and 4.5). In contrast, increase in visual colour score accompanied by decreasing carotenoids was noticed in ripe fruit despite of storage atmospheres (Figure 4.14 and 4.15). CA storage could retard colour development in 'Kensington Pride' mango (McLauchlan and Barker, 1992), but promoted colour development during ripening of 'Delta R2E2' mango (Lalel et al., 2005). The inferior colour development may be due to the inhibition action of CA and low temperature on carotenoid pigments formation (Teixeira, 2011; Singh and Singh, 2012). The retardation of 'Palmer' mango fruit colour development during CA storage has also been reported by Texeira and Durigan (2011). The increasing yellowness of visual colour may be due to the carotenoids levels in mango pulp (Figure 4.8 and 4.18) as reported by Vásques-Caicedo et al (2005).

4.4.3. Sugars and organic acids

In green mature 'Kensington Pride' mango, fructose was the predominant sugar, followed by sucrose, and glucose which was initially undetected. The sugar

composition of 'Kensington Pride' mango at harvest was similar to 'Irwin' mango (Ito et al., 1997). All sugars increased to the maximum value in all CA-stored fruit at week 3 and remained higher compared to NA-stored fruit at week 4 (Figure 4.6). This circumstance demonstrated CA condition can delay ripening process and sugars synthesis but not prevent these processes. Sucrose increased during ripening (Castrillo et al., 1992) and was reported as the main sugar in the ripe mango (Ito et al., 1997; Mitra and Baldwin, 1997) followed by fructose as the major reducing sugar (Castrillo et al., 1992; Ito et al., 1997). Huykens-Keil et al. (2006) also reported that the CA condition did not affect the carbohydrate metabolism of pepino fruit during storage.

The major sugar upon the retrieval of mango fruit from all storage conditions was sucrose, suggesting that the fruit were ripened during storage (Castrillo et al., 1992; Ito et al., 1997). Sucrose level in all fruit reduced during ripening at ambient temperature but was the highest contributor in total sugars, followed by fructose and glucose (Figure 4.16). However, the sucrose level in fruit from CA storage was lower than those from NA storage and conversely the level of glucose, showing the residual effects of CA storage. Several studies on different mango cultivars revealed that different cultivars can have different levels of sugars (Mitra and Baldwin, 1997) and CA conditions may have impeded the glycolytic pathway during ripening as reported by Burg (2004).

Succinic acid was found to be the major organic acid in 'Kensington Pride' mango, followed by citric and malic acid. This composition was different from 'Irwin' mango in which citric acid was reported as the major acid (Ito et al., 1997). Citric acid, the second major acid, increased to a maximum level at week 2 regardless of the atmosphere environment during storage, but there were no significant differences among all CA-stored fruit. The decreasing amount of citric acid was recorded in all treatments after reaching the peak at week 2, and NA-stored fruit showed the highest loss. Malic acid in CA-stored fruit was more stable than in NA-stored fruit since there were no significant changes in all CA-stored fruit within 4 weeks storage. The succinic acid in all CA-stored remained high after 4 weeks of storage (Figure 4.7). In total, all organic acids in CA-stored fruit was higher than in NA-stored fruit at 4 weeks storage, indicated that CO_2 inhibited the consumption of the TCA cycle intermediates (Mir and Beaudry, 2002).

After 4 weeks of storage, fruit ripened at ambient temperature and normal atmosphere for 4 days showed decreased amount of citric and succinic acids, whereas no significant changes in malic acid regardless of the storage conditions. This may be related to the nature of the temporary inhibition effects of CA storage on the respiration and ripening process. When the fruit regained their normal metabolism, the TCA cycle process returned to normal and the enzymes involved in this process resumed their activity (Mir and Beaudry, 2002). Decreased in levels of citric and succinic acid during ripening of mango fruit has also been reported by Lizada (1993).

4.4.4. Pulp carotenoids, ascorbic acid, and total antioxidants levels

CA storage suppressed the carotenoids synthesis during storage which was in accordance with the previous work of Lalel et al (2001). On the other hand, high rate of carotenoids synthesis but low carotenoids level was noticed in all CA-stored fruit after 4 days in ripening (Figure 4.18). Brecht et al (2003) reported that the development of the pulp colour was resumed in CA-stored 'Keitt' mango ripened in air for three days. Carotenoids in mango pulp may have been reflected as the visual yellow colour of fruit during ripening (Vásques-Caicedo et al., 2005) due to the transparency of the skin when chlorophylls were degraded to pheophoride and skin carotenoids were oxidised (Kays and Paull, 2004).

CA storage did not affect significantly the ascorbic acid during storage (Figure 4.9) but during ripening (Figure 4.19). All fruit from CA storage maintained high level of ascorbic acid in ripening. These results suggested that CA conditions preserved the ascorbic acid better than NA, either during storage or ripening of 'Kensington Pride' mango which were in contrast to the general trend in mango reported by Tefera et al (2007).

The total antioxidant levels decreased significantly during storage of mango fruit in NA and CA consisted of 3% O_2 with 5% or 6% CO_2 (Figure 4.10). However, when CA-stored fruit were ripened under normal air at 20°C, no significant effects of storage atmosphere and ripening time were observed (Figure 4.20). Phenolic compounds, ascorbic acid, carotenoids, and enzymes with antioxidant activity are known as antioxidants in dietary plants (Kondo et al., 2005; Ribeiro et al., 2008).

In conclusion, CA storage delayed fruit softening, green colour and chlorophylls degradations in fruit skin. CA storage delayed carotenoids formation in skin and pulp tissue during storage and through the ripening process of 'Kensington Pride' mango fruit. High sugars and organic acids concentrations were present after 4 weeks in CA storage and 4 days ripening. CA storage effectively hampered the activity of *exo*- and *endo*-PG until 3 weeks and reduced PE activity up to 4 weeks of storage, although it did not delay PG and PE during 4 days of fruit ripening. CA storage preserved ascorbic acid during mango fruit ripening.

Chapter 5

Effects of methyl jasmonate on softening, colour development, and quality of 'Kensington Pride' mango fruit during controlled atmosphere storage

Summary

The effects of methyl jasmonate (MJ) on softening, colour development and quality of 'Kensington Pride' mango fruit during controlled atmosphere (CA) storage were investigated. Hard mature green 'Kensington Pride' mango (*Mangifera indica* L.) fruit were vaporized with 0.01 mM MJ for 12 hours at 13 ± 1°C and stored at 13 ± 0.5°C, or in 90-L chamber of CA containing 3% O_2 and 5% CO_2 at 13 ± 0.5°C and 85 ± 3% RH for 6 weeks. The fruit were removed from storage for evaluations at 2-week intervals. Fruit texture profile including firmness, softening enzymes such as *exo*-, *endo*-Polygalacturonase (*exo*-, *endo*-PG), and pectin esterase (PE) activities, fruit skin colour and pigments, pulp sugars, organic acids, and nutritional quality were measured and analysed. The pre-CA storage application of MJ reduced the fruit firmness together with the *exo*-PG activity, and total organic acids, and maintained skin carotenoids. Together with CA storage, MJ treatment also increased levels of sugars in mango pulp, preserved chlorophylls hence delayed degreening in fruit skin during four weeks storage. The results suggested that MJ treatment combined with CA storage was beneficial in delaying colour development until four weeks and improving sugars to acids ratio.

5.1.Introduction

The mango fruit, like other climacteric fruit, ripens rapidly after harvest. The short postharvest life of mango limits its export potential to distant markets (Mitra and Baldwin, 1997; Yahia, 1998; Singh and Singh, 2012). Sea freight is not recommended for transporting the short life produce such as mango (Yahia, 1998), but quick shipment by air freight is limited due to higher costs (Illeperuma and Jayasuriya, 2002).

Jasmonates (jasmonic acid (JA) and methyl jasmonate (MJ)) are a class of oxylipins (Fan et al., 1998b, Khan et al., 2015). These are distributed in a variety of plant organs with fruit as the main pool (Meyer et al., 1984). They are considered as naturally occurring plant growth regulators (Creelman and Mullet, 1995), including cell division and fruit growth (Kondo and Fukuda, 2001), ripening (Fan et al., 1998b; Lalel et al., 2003g), and senescence (Ananieva et al., 2007). Methyl jasmonate inhibits microbial growth and decay both in intact and in fresh-cut fruit and vegetables (Buta and Moline, 1998; Cao et al., 2008; Creelman and Mullet, 1995). In mango fruit cv. 'Kensington Pride', the MJ concentration was reported to be high at harvest and decreased during ripening (Lalel et al,. 2003g). In contrast, JA increased and accumulated during storage and senescence have been reported in mango fruit cv 'Nam Dok Mai and 'Nang Klangwan' (Kondo et al., 2004b). The exogenous application of methyl jasmonate was proven to increase the ethylene production of climacteric fruit (Fan et al., 1998b) including 'Kensington Pride' mango (Lalel et al., 2003g) hence induced the ripening process.

Controlled atmosphere (CA) storage combined with an optimum storage temperature has been reported to extend shelf life and maintain the quality of different tropical fruits (Kader, 1992), including different mango cultivars (Abdulah and Basiouny, 2000; Dang et al., 2008b; Kim et al., 2007; Lalel et al., 2005; Lalel et al., 2006; Lizana and Ochagavia, 1996; Maekawa, 1990; Yahia and Hernandez, 1993). CA storage conditions regulateactivities of ripening related enzymes (Kanellis et al., 1989; Thompson, 1998) and TCA cycle enzymes including succinate dehydrogenase (Ke et al., 1993; Srilaong et al., 2006), resulted in abnormal ripening of fruit (Mitra and Baldwin, 1997) with higher concentration of succinic acid (Cadwallader, 2005).

The combined application of MJ with other treatments has been reported to improve the postharvest quality of several crops. The combination of MJ and 1-methylcyclopropene (1-MCP) treatments inhibited the production of volatile alcohols and esters in apple (Fan and Mattheis, 1999) which was in contrast to those resulted from the combination of MJ and ethephon treatments (Kondo et al., 2005b). Hot air treatment combined with MJ vapour resulted in lessens the chilling injury and maintains the quality of peach fruit (Jin et al., 2009). Gonzalez-Aguilar et al. (2003) reported that the postharvest quality of papaya 'Sunrise' was enhanced without any

off-flavour development when MJ was introduced in modified atmosphere packaging.

Delay in ripening is necessary in order to extend the storage life of horticulture products. However, the application of CA storage has shown the residual effects that disturb the ripening process which impact the quality of the produce after storage (Cadwallader, 2005; Kanellis et al., 1989; Ke et al., 1993; Mitra and Baldwin, 1997; Srilaong et al., 2006; Thompson, 1998). Poor skin colour development on fruit, high acidity, and fruit softening are coupled with CA storage and sea freight (Anonymous 2002; Singh and Zaharah, 2015). Lack of information in enhancing the mango fruit quality in CA storage limits the application of this technology to prolong the storage life. It was hypothesized that the combination of MJ as the ripening promoter and the CA storage as the ripening depressor could be more effective in managing the quality of CA-stored mango. The objective of this study was to elucidate the effects of MJ treatment in improving the physico-chemical quality of 'Kensington Pride' mango during CA storage.

5.2. Materials and methods

5.2.1. Fruit and experimental conditions

Hard mature green 'Kensington Pride' mangoes were sourced from a commercial orchard located at Chittering (long. 116°5'E, lat. 31°25'S), Western Australia in March 2007. Fruit were dipped for 2 minutes in an aqueous fungicide solution (0.55 $ml \cdot l^{-1}$ 'Sportak'), which contained prochloraz as an active ingredient), air dried, packed in the soft-board, and transported by refrigerated vehicle (15 ± 1°C) to Perth, Western Australia. Uniformly mature fruit, free from visual symptoms of any diseases or blemishes were chosen for the experiment.

The experiment was designed by following a completely randomised design. Three lots of 10 fruit were incubated in the 90-l airtight chambers for 12 hours together with filter paper spotted with methyl jasmonate (MJ) (100%) to achieve a vapour concentration of 0.01 mM. Another three lots of 10 fruit were also incubated in the 90-l airtight chambers for 12 hours without MJ vapour. After 12 hours incubation at 13 ± 0.5°C, the fruit were stored in normal atmosphere (NA) and in 90-l chambers of controlled atmosphere (CA) containing 3% O_2 and 5% CO_2 at 13 ± 0.5°C and 85 ± 3% RH. Concentration of O_2 and CO_2 in the CA chambers were

adjusted with N_2. The CA storage conditions were maintained as described in section 4.2.1.1. A single chamber containing 10 fruit was treated as one treatment unit and replicated three times. The fruit were removed after 2, 4, and 6 weeks of storage.

5.2.2. Measurement of fruit texture profile

Fruit were cut at the equatorial area of the fruit (2 x 2 cm^2 in width x height) and the texture profiles were analysed by texture analyser as previously described in section 3.3.2.2.

5.2.3. Fruit softening enzyme activity analysis

The activity of fruit softening enzymes i.e., *exo*-polygalacturonase (*exo*-PG), *endo*-polygalacturonase (*endo*-PG), and pectin esterase (PE) were determined as detailed in section 3.4.

5.2.3.1. Extraction and determination of pectolytic enzymes activity

The pectolytic enzymes activity, including *exo*- and *endo*-PG, and PE activities, from pulp tissues were extracted and determined as detailed in chapter 3 (sections 3.4.1 and 3.4.2).

5.2.3.2. Protein analysis

Protein content from pulp tissue was determined following the method of Bradford (1976) with some modifications as described in section 3.4.3.

5.2.4. Fruit colour assessment

Visual and Hunter scale measurements were used in assessing the fruit skin colour. Individual fruit from each replication was evaluated during CA storage of control and MJ-treated samples. Subjective and objective skin colour were determined as detailed in sections 3.5.1 and 3.5.2.

5.2.5. Skin pigment analysis

Skin pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) were measured as described in section 3.6.

5.2.6. Sugars and organic acids analysis

High performance liquid chromatography (HPLC Waters, Milford, MA, USA) was used in sugars and organic acids determinations as detailed in section 3.7.1 and 3.7.2.

5.2.7. Pulp total carotenoids analysis

Mango pulp total carotenoids were estimated according to the method of Tomes (1963) and Lalel (2002) as described in section 3.8.

5.2.8. Ascorbic acid analysis

The concentration of fruit pulp ascorbic acid was determined as described in section 3.9.

5.2.9. Determination of total antioxidants

Total antioxidant activity was determined as described in section 3.10.

5.2.10. Statistical analysis

Effects of the MJ treatment combined with CA storage on fruit softening, colour changes, and nutritive value of 'Kensington Pride' mango were assessed as described in section 3.11.

5.3. Results

5.3.1. Texture profiles

Storage treatment significantly affected ($P \leq 0.05$) fruit firmness, springiness, and adhesive force, but not cohesiveness, gumminess, and fracture force. On the other hand, storage period significantly affected ($P \leq 0.05$) fruit texture profile measured except fracture force (Table 5.1). Fruit firmness decreased up to 90% after 2 weeks storage with or without MJ treatment in normal atmosphere storage. Fruit stored in CA for 2 weeks also showed significant reduction in firmness, however MJ-treated fruit stored in CA exhibited significantly higher reduction (86%) compared to those without MJ treatment (83%). Further loss in fruit firmness was noticed in all treatments at 4 weeks storage although no significant differences were found between control and MJ-treated fruit stored in normal atmosphere. When fruit stored in CA for 4 weeks, the firmness was significantly higher than MJ-treated fruit stored in CA (Table 5.1). No significant loss in fruit firmness when storage period

extended to 6 weeks, except MJ-treated fruit stored in CA. At 6 weeks storage, control fruit stored in CA was firmer than MJ-treated fruit stored in normal atmosphere, but not significantly different from other storage treatments. Similarly, there were no significant differences among MJ-treated and untreated fruit stored in normal and controlled atmosphere, and control fruit stored in normal atmosphere.

In general, springiness was reduced during storage and was significantly affected by storage treatment and storage period. Methyl jasmonate treated fruit stored under normal atmosphere exhibited the highest decrease in springiness (Table 5.1). Adhesive force was also affected by both storage treatment and storage period. Control and MJ-treated fruit stored in CA always showed higher adhesive force compared to those stored under normal atmosphere, regardless of the storage period. Changes in cohesiveness and gumminess were not significantly affected by storage treatments but storage period. In both cases, the lowest value was demonstrated in MJ-treated fruit stored in normal atmosphere for 6 weeks.

5.3.2. Fruit softening enzymes

5.3.2.1. *Exo*- and *endo*-polygalacturonase enzyme activities

Different storage treatment and storage period significantly ($P \leq 0.05$) affected *exo*-polygalacturonase enzyme activity. On the other hand, only storage period significantly ($P \leq 0.05$) affected *endo*-polygalacturonase enzyme activity (Table 5.2). The *exo*-PG activity increased during 4 weeks storage, but significantly declined at week 6 regardless of the storage treatment. The highest increase was noticed in CA stored fruit without MJ treatment at 4 weeks storage (Figure 5.1). No significant differences were noticed between MJ treated fruit stored under normal and controlled atmosphere for 6 weeks.

The *endo*-PG activity, measured as changes in viscosity, of control and MJ-treated fruit decreased significantly ($P \leq 0.05$) at week 4, whereas CA-stored, and MJ-treated combined with CA storage decreased significantly at week 2. There were no differences between MJ-treated fruit stored in normal and controlled atmosphere at week 6. Similarly, no significant differences were exhibited between control fruit stored under normal and controlled atmosphere for 6 weeks (Figure 5.1). The fruit firmness showed a significant ($P \leq 0.0001$) positive correlation with *endo*-PG activity ($r = 0.8374$) but no correlation with *exo*-PG activity (Table 5.3).

Table 5.1. Effects of methyl jasmonate and CA on the texture profile during storage of 'Kensington Pride' mango fruit at 13°C

Treatments	Firmness (N)								*Means* (SP)	LSD ($P \leq 0.05$)
	Storage period (week)									
	0		2		4		6			
Control	124.21	aA	7.87	bC	5.18	cC	4.83	cAB	*35.52*	T = 0.653
CA	124.21	aA	21.49	bA	8.14	cA	5.49	dA	*39.83*	SP = 0.653
MJ + NA	124.21	aA	7.75	bC	5.25	cC	4.06	cB	*35.32*	T x SP =
MJ + CA	124.21	aA	16.87	bB	7.16	cB	5.04	dAB	*38.32*	1.306
Means (T)	*124.2*		*13.49*		*6.43*		*4.86*		*37.25*	
Cohesiveness										
Control	0.059	aA	0.034	bB	0.045	aAB	0.036	aB	*0.043*	T = ns
CA	0.059	aA	0.067	aA	0.052	aA	0.026	ab	*0.051*	SP = 0.0108
MJ + NA	0.059	aA	0.038	bAB	0.052	aAB	0.018	bB	*0.042*	T x SP = ns
MJ + CA	0.059	aA	0.046	bA	0.052	aA	0.022	bB	*0.045*	
Means (T)	*0.059*		*0.046*		*0.050*		*0.026*		*0.045*	
Springiness (N)										
Control	3.005	aA	1.282	cC	1.577	bB	1.202	cA	*1.766*	T = 0.212
CA	3.005	aA	2.405	bA	2.102	cA	1.122	dB	*2.158*	SP = 0.212
MJ + NA	3.005	aA	1.456	bC	1.432	bB	0.825	cB	*1.680*	T x SP = ns
MJ + CA	3.005	aA	1.816	bB	1.914	bA	1.011	cB	*1.936*	
Means (T)	*3.005*		*1.740*		*1.756*		*1.040*		*1.885*	
Gumminess (N)										
Control	7.330	aA	0.280	bBC	0.230	bA	0.170	bA	*2.010*	T = ns
CA	7.330	aA	1.510	bA	0.420	bA	0.140	bA	*2.350*	SP = 0.746
MJ + NA	7.330	aA	0.310	bAB	0.260	bA	0.080	bA	*1.990*	T x SP = ns
MJ + CA	7.330	aA	0.850	bAB	0.380	bA	0.110	bA	*2.170*	
Means (T)	*7.330*		*0.740*		*0.320*		*0.130*		*2.130*	
Fracture force (N)										
Control	1.52	aA	0.51	bA	0.76	abA	0.71	bA	*0.88*	T = ns
CA	1.52	aA	5.15	aA	0.57	aA	0.55	aA	*1.95*	SP = ns
MJ + NA	1.52	aA	0.99	abA	0.49	bA	0.97	bA	*0.99*	T x SP = ns
MJ + CA	1.52	aA	0.65	bA	0.57	bA	0.84	abA	*0.9*	
Means (T)	*1.52*		*1.82*		*0.6*		*0.77*		*1.18*	
Adhesive force (N)										
Control	0.623	aA	0.536	aC	0.362	bB	0.668	aC	*0.547*	T = 0.102
CA	0.623	bA	1.022	aA	0.573	bA	0.809	abBC	*0.757*	SP = 0.102
MJ + NA	0.623	bA	0.649	bBC	0.145	cC	0.957	aAB	*0.593*	T x SP =
MJ + CA	0.623	bcA	0.891	abA	0.521	cAB	1.111	aA	*0.787*	0.203
Means (T)	*0.623*		*0.774*		*0.4*		*0.89*		*0.671*	

n = 30 (10 fruit x 3 replications), ns = not significant at $P \leq 0.05$. CA = controlled atmosphere (3% O_2 and 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate, T = treatment, SP = storage period. Values within a row followed by the same lowercase letter(s) are not significantly different and values within a column followed by the same uppercase letter(s) are not significantly different at $P \leq 0.05$.

5.3.2.2. Pectin esterase enzyme activity

Storage period significantly affected the PE enzyme activity, but there were no significant effects of storage treatment and the interactions between storage treatment and storage period at $P \leq 0.05$ (Table 5.2). All fruit exhibited significant reduction in PE activity after 2 weeks storage and no further significant changes toward the end of six weeks storage period. After six weeks storage, no significant differences among MJ-treated stored either under normal or controlled atmosphere and CA-stored fruit without MJ treatment (Figure 5.1). Also, no significant differences between control fruit stored under normal and controlled atmosphere at 6 weeks storage. The fruit firmness showed a significant ($P \leq 0.0001$) positive correlation with PE activity (Table 5.3).

Table 5.2. Effects of methyl jasmonate and CA on pectolytic enzymes during storage of 'Kesington Pride' mango fruit at 13°C

Treatments	*Exo*-PG (µg GalA·mg prot^{-1}·h^{-1}) Storage period (weeks)								*Means* (SP)	LSD ($P \leq 0.05$)
	0		2		4		6			
Control	87.4	bcA	73.3	cB	146.2	aB	112.6	abB	*104.9*	T = 14.78
CA	87.4	cA	66.4	cB	237.6	aA	128	bA	*129.9*	SP = 14.78
MJ + NA	87.4	bA	69.3	bB	141.2	aB	1	cC	*74.7*	T x SP =
MJ + CA	87.4	bA	105.5	bA	153.2	aB	1.3	cC	*86.9*	29.57
Means (T)	*87.4*		*78.6*		*169.6*		*60.7*		*99.1*	
Endo-PG (Viscosity changes in sec·mg prot^{-1}·h^{-1})										
Control	15.63	aA	8.83	bA	0.01	cC	0.63	cB	*6.27*	T = ns
CA	15.63	aA	0.74	bB	1.84	bA	0.01	bB	*4.56*	SP = 2.74
MJ + NA	15.63	aA	7.65	bA	1.54	bA	2.19	bA	*6.75*	T x SP = ns
MJ + CA	15.63	aA	0.54	bB	0.93	bB	2.86	bA	*4.99*	
Means (T)	*15.63*		*4.44*		*1.08*		*1.42*		*5.64*	
PE (mM NaOH·mg prot^{-1}·h^{-1})										
Control	0.18	aA	0.015	bC	0.039	bA	0.016	bB	*0.061*	T = ns
CA	0.18	aA	0.017	bBC	0.043	bA	0.019	bAB	*0.063*	SP = 0.017
MJ + NA	0.18	aA	0.022	bAB	0.027	bA	0.025	bA	*0.062*	T x SP = ns
MJ + CA	0.18	aA	0.028	bA	0.032	bAB	0.026	bA	*0.065*	
Means (T)	*0.18*		*0.021*		*0.036*		*0.021*		*0.063*	

n = 3 replications, ns = not significant at $P \leq 0.05$. CA = controlled atmosphere (3% O_2 and 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate, T = treatment, SP = storage period. Values within a row followed by the same lowercase letter(s) are not significantly different and values within a column followed by the same uppercase letter(s) are not significantly different at $P \leq 0.05$.

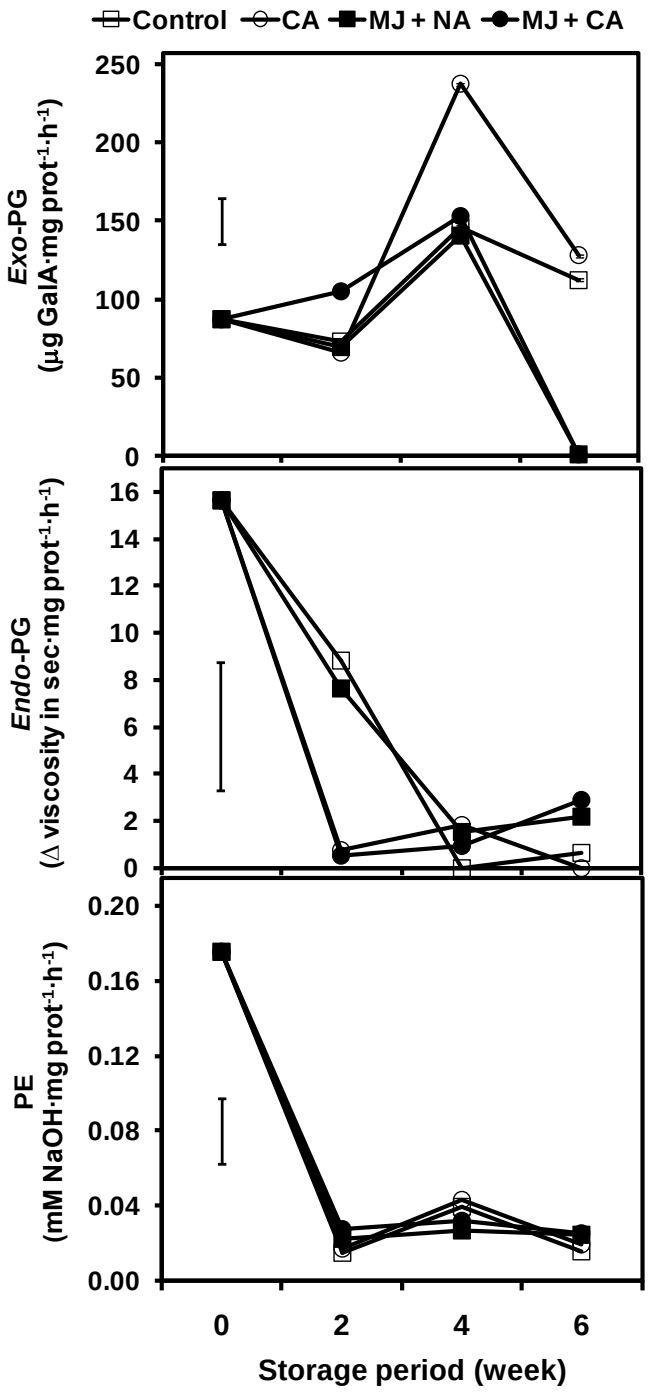

Figure 5.1. Effects of MJ and CA (T) on the activity of *exo*-PG, *endo*-PG, and PE enzymes during 6 weeks of storage period (SP) at 13°C. Vertical bar represents LSD ($P \leq 0.05$). LSD ($P \leq 0.05$) for *exo*-PG: T = 14.78, SP = 14.78, T x SP = 29.57; *endo*-PG: T = ns, SP = 2.74, T x SP = ns; PE: T = ns, SP = 17.43, T x SP = ns. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate. ns = not significant at $P \leq 0.05$.

Table 5.3. Relationship between fruit firmness and fruit softening enzymes in pulp tissues of 'Kensington Pride' mango treated with MJ during storage at 13°C

Variable compared	Pearson's correlation coefficients (r)
Firmness vs *Exo*-polygalacturonase	-0.118**
Firmness vs *Endo*-polygalacturonase	0.837***
Firmness vs Pectin esterase	0.957***

, * = significant at $P \leq 0.001$ or $P \leq 0.0001$ respectively.

5.3.3. Fruit colour

Storage treatment and storage period significantly ($P \leq 0.05$) affected the overall fruit skin chromaticity values. The L*, a, b, and chroma values of fruit stored in NA, treated with or without MJ, were significantly increased during 6 weeks of storage, whereas those stored in CA were almost unchanged (Figure 5.2). Similar trend was noticed for the visual colour of the skin, i.e. CA treatments, with or without MJ, slowed down the loss of green colour during storage. On the other hand, the skin of this fruit exhibited a reverse trend of hue angle as the storage proceeded. The L* value of control and MJ-treated fruit increased up to 25% but only 1% to 3% changes were noticed in CA stored fruit, both with and without MJ treatment. The a* value of fruit stored under normal atmosphere (control and MJ-treated fruit) were improved more than 100% from their initial a* values which were in contrast to those stored under CA storage, either with or without MJ treatment. In MJ-treated fruit, the b* value improved the most (42%) although they were not different from control ones. Contrarily, the b* value of fruit stored under CA storage was almost unchanged and it was not significantly different from fruit stored under the same atmosphere combined with MJ treatment after 6 weeks of storage. There was about 38% increase in chroma value of control and MJ-treated fruit stored under normal atmosphere, but no increments in all fruit stored under CA storage for 6 weeks. The hue angle of control and MJ-treated fruit decreased more than 25% from their initial values, while there were no changes in all CA-stored fruit after 6 weeks of storage. The CA storage, with or without MJ treatment, slowed down the loss of green colour (as indicated by the increasing values of colour ratings as storage time progressed) compared to the NA treatments. That suggests that MJ application had little impact

on skin colour development from green to yellow during storage, as opposed to CA conditions.

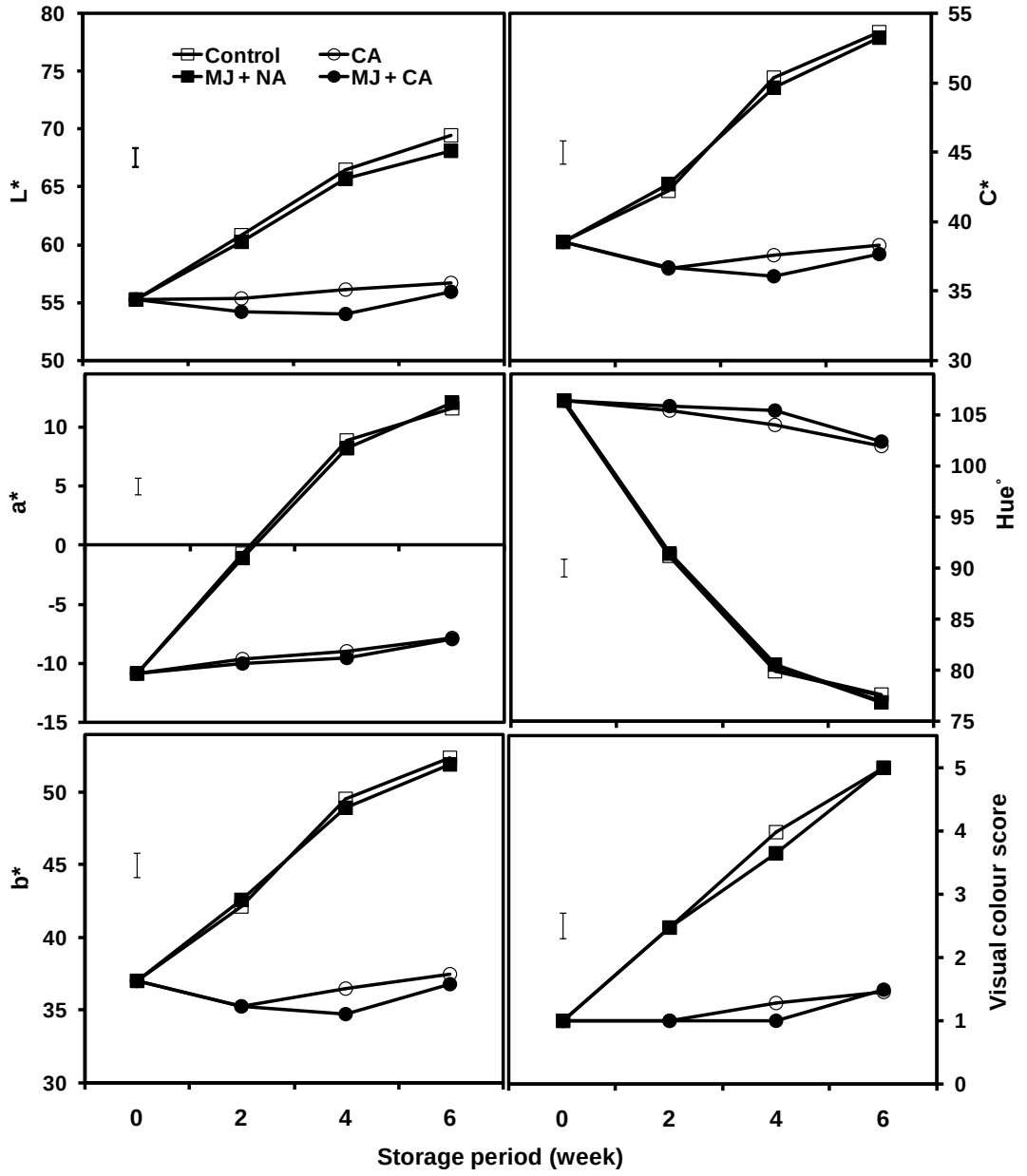

Figure 5.2. Effects of MJ and CA (T) on skin colour of mango fruit during 6 weeks of storage period (SP) at 13°C. Vertical bars represent LSD ($P \leq 0.05$). LSD ($P \leq 0.05$) for L*: T = 0.81, SP = 0.81, T x SP = 1.62; a*: T = 0.68, SP = 0.68, T = 1.35; b*: T = 0.86, SP = 0.86, T x SP = 1.72; C*: T = 0.86, SP = 0.86, T x SP = 1.72; Hue°: T = 0.86, SP = 0.86; T x SP = 1.72; visual colour: T = 0.20, SP = 0.20, T x SP = 0.40. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate.

5.3.4. Fruit skin pigments

Storage treatment and storage period significantly ($P \leq 0.05$) influenced the skin pigments of 'Kensington Pride' mango. Control and MJ-treated fruit exhibited chlorophyll loss (48% and 70%, respectively) during 2 weeks of storage, while CA-stored and MJ-treated combined with CA storage lost only 10% and 20%, respectively, during the same period. The chlorophylls continued to reduce until 6 weeks of storage regardless of the storage treatments. More than 90% of total chlorophylls were lost in control and MJ-treated fruit stored under normal atmosphere compared to less than 50% lost in fruit stored under CA, with or without MJ treatment at the end of the observed storage period. That suggests that CA conditions had a strong impact on skin pigments degradation during storage compared to MJ application.

As the storage proceeded for 2 weeks, the carotenoids levels of control and MJ-treated fruit increased significantly from 22.96 $\mu g \cdot g^{-1}$ FW skin to 41.1 and 37.5 $\mu g \cdot g^{-1}$ FW skin, respectively, whereas CA-stored fruit, both with and without MJ treatment, remained stable. However, the skin of control fruit and CA-stored without MJ treatment fruit encountered severe lost in carotenoids content at 4 weeks of storage. Contrarily, MJ-treated fruit continued to synthesize carotenoids (37.5 to 47.71 $\mu g \cdot g^{-1}$ FW skin) and those stored under CA storage remained stable. There was loss of carotenoids in MJ-treated fruit (both stored in normal and controlled atmosphere) at 6 weeks of storage (Figure 5.3).

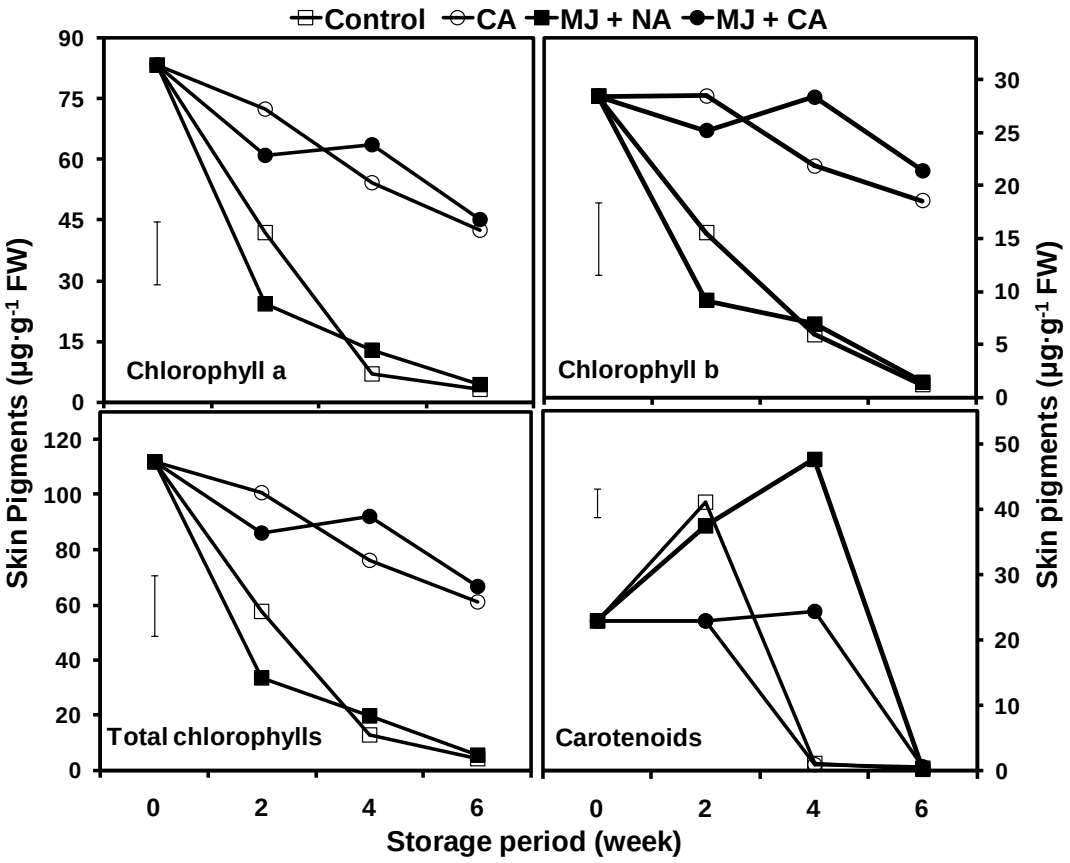

Figure 5.3. Effects of MJ and CA (T) on skin pigments of mango fruit during 6 weeks of storage period (SP) at 13°C. Vertical bars represent LSD ($P \leq 0.05$). LSD ($P \leq 0.05$) for chlorophyll a: T = 7.78, SP = 7.78, T x SP = 15.57; chlorophyll b: T = 3.38, SP = 3.38, T = 6.75; total chlorophylls: T = 10.96, SP = 10.96, T x SP = 21.93; carotenoids: T = 2.13, SP = 2.13, T x SP = 4.25. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate.

5.3.5. Sugars and organic acids

5.3.5.1. Sugars

Storage treatments had significant ($P \leq 0.05$) effect on glucose levels but not on sucrose, fructose and total sugars. Storage period significantly ($P \leq 0.05$) influenced sucrose, glucose, fructose and total sugars in mango fruit. After 2 weeks in storage, sucrose increased significantly in control, CA storage, MJ-treated, but not in MJ-treated combined with CA storage fruit. The highest increased in sucrose levels was quantified in MJ-treated fruit (about 4 folds), followed by control fruit (3

folds), and then CA-treated fruit (2 folds) from the initial concentration of 19.03 to 82.80, 64.09, and 49.03 $\mu g \cdot mg^{-1}$ FW, respectively. Sucrose level in MJ-treated combined with CA storage fruit increased after 4 weeks in storage, whereas other treatments showed a decreasing trend toward the end of storage observation. MJ-treated combined with CA storage fruit contained the highest sucrose compared to other treatments after 6 weeks of storage; however, it was not significantly different from CA-stored fruit without MJ treatment (Figure 5.4).

Glucose levels in control fruit, CA-stored fruit with and without MJ treatment increased significantly ($P \leq 0.05$) at week 2 of storage, i.e., 4, 5, and 9 folds from their initial's, respectively. Glucose content further increased at week 4 in CA-stored and MJ-treated fruit, but not in control fruit, before it remained steady up to week 6 in storage. The glucose content of MJ-treated fruit increased as much as 8 folds at the later storage period, but there were no further changes afterwards. Control fruit contained the lowest levels of glucose at week 6 of storage which was significantly low compared to other storage treatments (Figure 5.4).

Except in CA-stored fruit, the fructose level in other treatments increased ($P \leq 0.05$) at week 2. Fructose levels increased in control, MJ-treated, and MJ-treated combined with CA storage fruit, as much as 1.3, 1.6, and 2.1 fold, respectively. Fructose level in CA-stored fruit started to increase significantly at week 4 towards the end of the trial (Figure 5.4). Further storage of MJ-treated fruit resulted in increasing fructose level at week 6 whereas fruit of control and MJ combined with CA storage did not. After 6 weeks of storage, fructose level in control fruit was significantly lower compared to CA-stored and MJ-treated fruit (31.19 compared to 42.70 and 44.14 $\mu g \cdot mg^{-1}$ FW, respectively). On the other hand, there were no significant differences among CA-stored, MJ-treated, and MJ-treated combined with CA stored fruit.

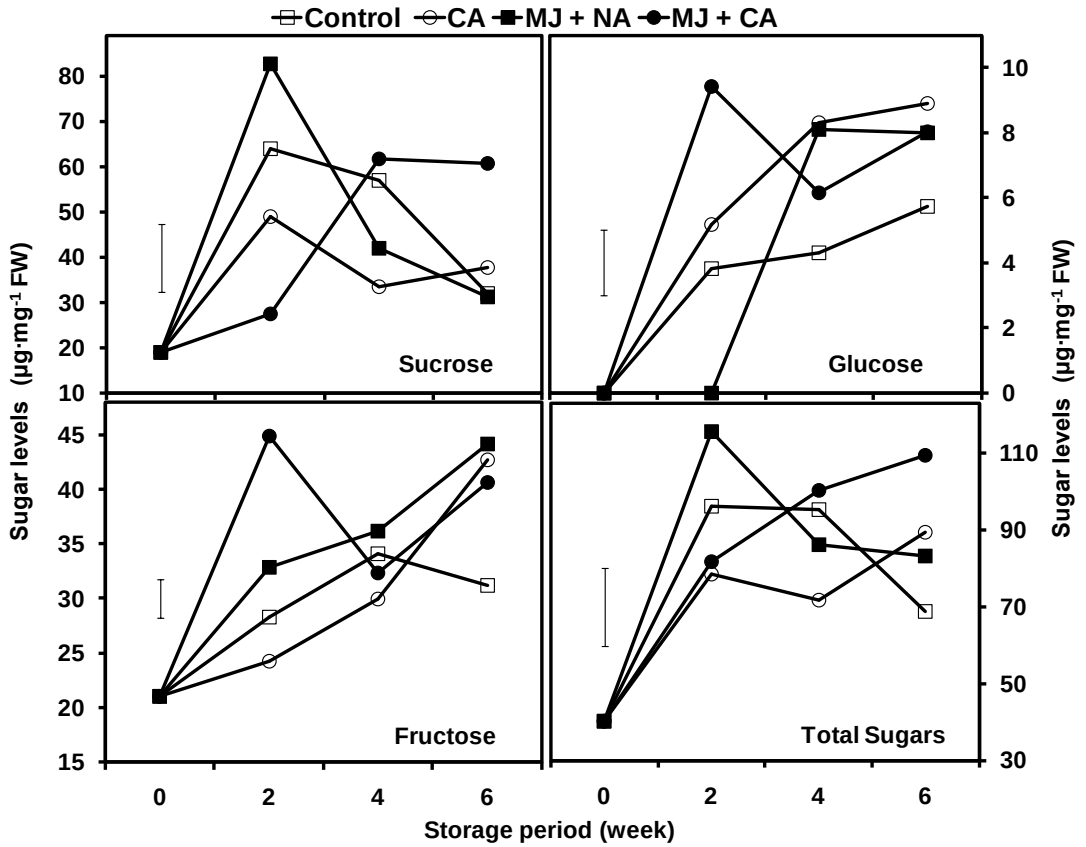

Figure 5.4. Effects of MJ and CA (T) on sugar levels in mango fruit during 6 weeks of storage period (SP) at 13°C. Vertical bars represent LSD ($P \leq 0.05$). LSD ($P \leq 0.05$) for sucrose: T = ns, SP = 7.49, T x SP = 14.97; glucose: T = 0.87, SP = 0.87, T = 1.73; fructose: T = ns, SP = 3.53, T x SP = 7.07; Total sugars: T = ns, SP = 10.28, T x SP = ns. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate. ns = not significant.

The percentage of sucrose in fruit before storage was lower than fructose (47 : 52). As the storage proceeded to week 2, the sucrose levels increased almost double compared to fructose in most treatments, except MJ-treated combined with CA storage. However, the proportions of sucrose and fructose in control and CA-treated fruit were almost equal (46 : 45 and 42 : 48, respectively) after 6 weeks of storage. Contrarily, the amount of sucrose and fructose in MJ-treated fruit, either with or without CA storage, was in reverse from week 2. The sucrose to fructose ratio of MJ-treated fruit was 37 : 53, and that of MJ combined with CA storage was 56 : 37.

5.3.5.2.Organic acids concentrations

Storage treatment, storage period and their interaction significantly ($P \leq 0.05$) influenced the organic acid concentrations in the fruit. Citric acid content reduced by 90% in MJ-treated fruit stored under normal and controlled atmosphere environment after 2 weeks of storage, whereas in control fruit it reduced by only 47% (Figure 5.5.). Further storage of those fruit did not significantly affect the citric acid concentration. At the final week of observation, the citric acid concentration in CA-stored fruit was the highest (30.58 $\mu g \cdot mg^{-1}$ FW) which was significantly higher than other storage treatments.

Malic acid concentration in control and CA-stored fruit increased significantly ($P \leq 0.05$) (more than 50%) after 4 weeks of storage. In contrast, malic acid concentration in MJ-treated fruit stored either under normal or controlled atmosphere reduced significantly (91% or 80%, respectively) after two weeks of storage, with no further reduction afterwards (Figure 5.5). At week 6 of storage, with CA-stored fruit showed the highest malic acid concentration, which was two times higher than control fruit and 10 times higher than MJ-treated fruit, regardless of the storage atmosphere

The concentration of succinic acids in control fruit did not show significant changes after 2 weeks storage period, whereas all MJ-treated fruit did (90% reduction) and remained steady to 6 weeks storage period. Contrarily, CA-stored fruit did not show significant reductions of succinic acid throughout the storage period (Figure 5.5). At the final week of observation, CA-stored fruit had the highest succinic acid (86.99 $\mu g \cdot mg^{-1}$ FW), followed by control fruit (75.95 $\mu g \cdot mg^{-1}$ FW), and then MJ-treated fruit stored either in normal or in CA (7.64 and 9.16 $\mu g \cdot mg^{-1}$ FW, respectively).

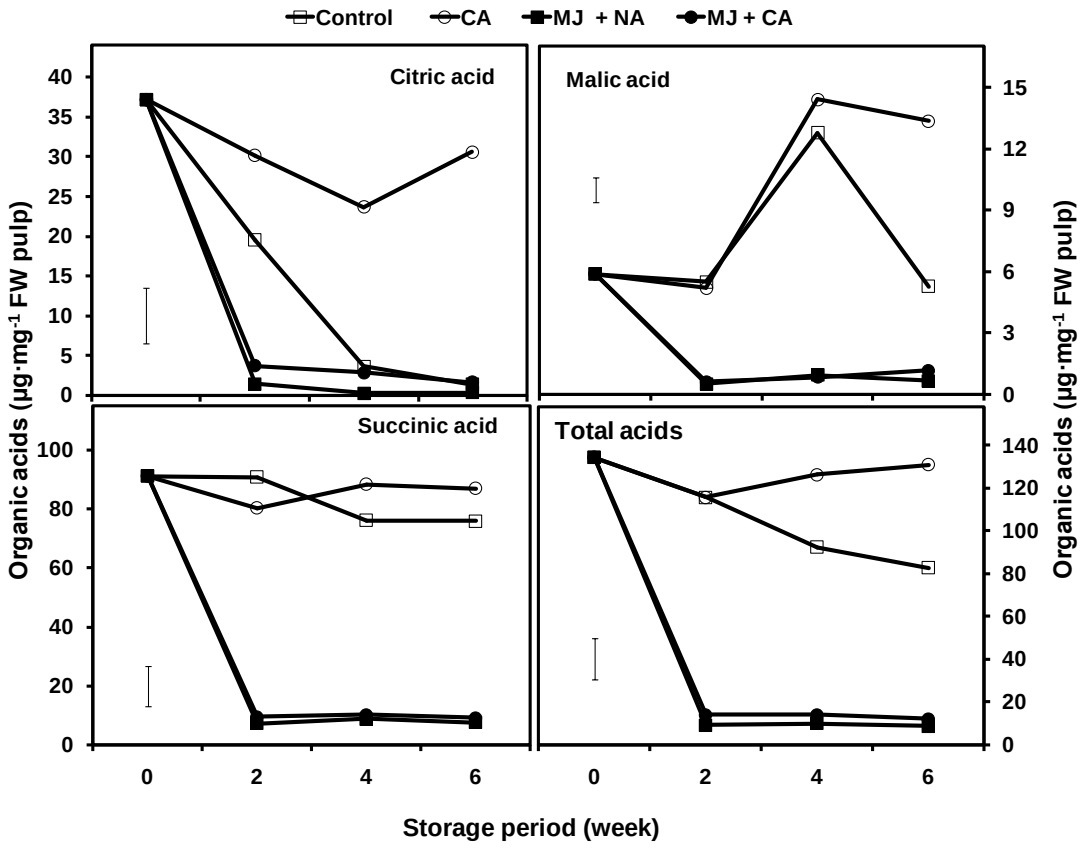

Figure 5.5. Effects of MJ and CA (T) on organic acids levels in mango fruit during 6 weeks of storage period (SP) at 13°C. Vertical bars represent LSD ($P \leq 0.05$). LSD ($P \leq 0.05$) for citric acid: T =3.46, SP = 3.46, T x SP = 6.92; malic acid: T = 0.61, SP = 0.61, T = 1.21; succinic acid: T = 6.87, SP = 6.87, T x SP = 13.74; Total acids: T = 9.79, SP = 9.79, T x SP = 19.57. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate.

In general, the compositions of the organic acids in 'Kensington Pride' mango prior to storage were 27% citric acid, 4% malic acid, and 23% of succinic acid. The total organic acid concentrations in all storage treatments declined as the storage proceeded to week 2, although there was no significant reduction in control and CA-stored fruit. Further storage resulted in significant decrease in organic acids content in control fruit, but not in all MJ-treated fruit stored under normal and controlled atmosphere. The highest contents of organic acids remained in CA-stored fruit (130.91 µg·mg^{-1} FW) followed by control (82.58 µg·mg^{-1} FW), MJ-treated fruit combined with CA storage (11.95 µg·mg^{-1} FW), then MJ-treated stored under normal atmosphere (8.63 µg·mg^{-1} FW) after 6 weeks of storage.

5.3.6. Carotenoids, ascorbic acid, and total antioxidants in mango pulp

Carotenoids and ascorbic acid levels in mango fruit were significantly ($P \leq 0.05$) affected by storage treatments and storage period (Figure 5.6 and 5.7). In contrast, total antioxidant content was not significantly affected by storage treatments but by storage period (Figure 5.8, Table 5.4).

CA-stored fruit treated with or without MJ, did not show significant change in pulp carotenoids concentration during storage, however, they were significantly lower compared to all fruit stored under normal atmosphere condition. The concentrations of carotenoids in fruit treated with MJ, regardless the atmosphere storage condition, increased significantly (94% and 80% in MJ-treated under normal and CA storage, respectively) at week 2 and remained constant towards week 6 in storage. The carotenoids levels in control fruit and CA-stored fruit increased significantly at week 2 (93% and 66%, respectively). There was further increase at week 4 (26% and 49% higher compared to week 2, respectively) before it remained steady until week 6 in storage (Figure 5.6.).

The ascorbic acid concentrations decreased significantly ($P \leq 0.05$) in all treatments during the 6 weeks of the trial (Figure 5.7). Fruit treated with MJ without CA storage had the highest loss in ascorbic acid at 2 and 6 weeks but at 4 weeks control fruit had the highest loss. Fruit treated with MJ without CA storage lost about 95% of ascorbic acid content compared to its initial level, followed by control (87%), MJ with CA storage (88%), and CA storage fruit (74%).

The levels of total antioxidants in mango fruit pulp decreased significantly ($P \leq 0.05$) during storage of MJ-treated fruit stored in normal atmosphere at 4 and 6 weeks of storage, but there were little differences for the other treatments or storage times. The mean level of total antioxidants in mango pulp was 3.88 μmol TE·100 g^{-1} FW before storage and decrease to 2.82 μmol TE·100 g^{-1} FW after 6 weeks of storage (Figure 5.9.). Total antioxidants content in MJ-treated fruit decreased significantly during 2 and 4 weeks and remained unchanged up to 6 weeks in CA storage.

Table 5.4. Effects of MJ and CA on carotenoids, ascorbic acid, and total antioxidants of 'Kensington Pride' mango during 6 weeks of storage at 13 ± 0.5°C and 85 ± 3% RH

Treatments	Carotenoids (µg·g^{-1} FW pulp)									*Means* (T)	LSD ($P \leq 0.05$)
	Storage period (weeks)										
	0			2			4			6				
Control	3.10	c	A	46.10	b	A	62.30	a	A	50.60	ab	A	*40.50*	T = 5.92
CA	3.10	c	A	9.10	b	B	17.80	a	B	21.00	a	B	*12.70*	SP = 5.92
MJ + NA	3.10	b	A	53.50	a	A	66.40	a	A	60.10	a	A	*45.77*	T x SP = 11.83
MJ + CA	3.10	b	A	16.00	a	B	20.00	a	B	17.80	a	B	*14.22*	
Means (SP)	*3.10*			*31.20*			*41.60*			*37.40*			*28.30*	
Ascorbic acid (mg·100 g^{-1} FW)														
Control	44.16	a	A	24.89	b	B	3.31	d	D	5.98	c	B	*19.58*	T = 0.785
CA	44.16	a	A	28.26	b	A	6.96	d	B	11.19	c	A	*22.64*	SP = 0.785
MJ + NA	44.16	a	A	7.57	b	D	5.17	c	C	2.16	d	C	*14.76*	T x SP = 1.571
MJ + CA	44.16	a	A	10.47	b	C	11.18	b	A	5.41	c	B	*17.8*	
Means (SP)	*44.16*			*17.79*			*6.65*			*6.19*			*18.7*	
Total antioxidants (µmol TE·100 g^{-1} FW)														
Control	3.878	a	A	3.447	a	A	2.92	a	A	3.109	a	AB	*3.339*	T = ns
CA	3.878	a	A	2.949	a	A	3.399	a	A	3.322	a	A	*3.387*	SP = 0.49
MJ + NA	3.878	a	A	3.776	a	A	2.204	b	B	1.747	b	B	*2.901*	T x SP = ns
MJ + CA	3.878	a	A	3.202	a	A	2.818	a	AB	3.107	a	AB	*3.251*	
Means (SP)	*3.878*			*3.343*			*2.835*			*2.821*			*3.22*	

n = 3 replications, ns = not significant at $P \leq 0.05$. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate, T = treatment, SP = storage period. Values within a row followed by the same lowercase letter(s) are not significantly different and values within a column followed by the same uppercase letter(s) are not significantly different at $P \leq 0.05$.

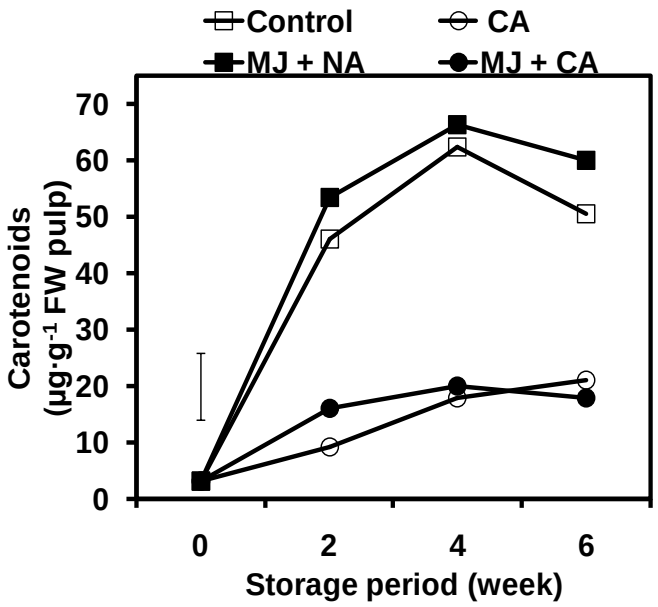

Figure 5.6. Effects of MJ and CA (T) on carotenoids in mango fruit pulp during 6 weeks of storage period (SP) at 13°C. Vertical bars represent LSD ($P \leq 0.05$). LSD ($P \leq 0.05$): T = 5.92, SP = 5.92, T x SP = 11.83. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate.

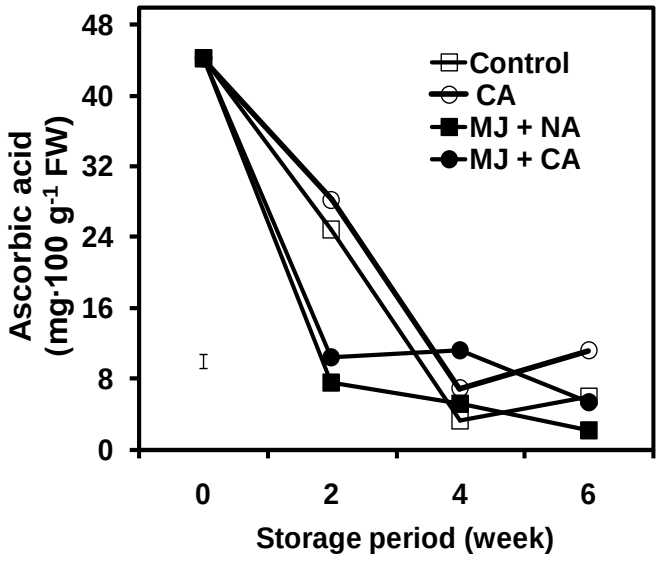

Figure 5.7. Effects of MJ and CA (T) on the levels of ascorbic acid in mango fruit pulp during 6 weeks of storage period (SP) at 13°C. Vertical bars represent LSD ($P \leq 0.05$). LSD ($P \leq 0.05$): T = 0.79, SP = 0.79, T x SP = 1.57. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate.

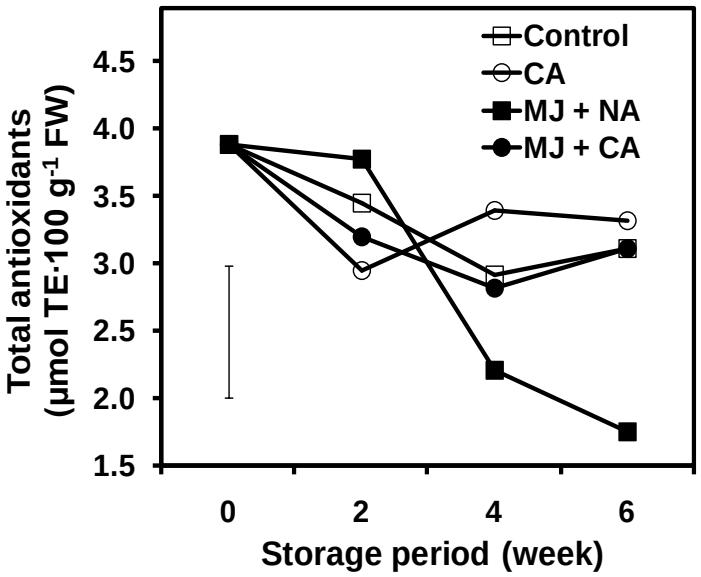

Figure 5.8. Effects of MJ and CA (T) on the levels of total antioxidants in mango fruit pulp during 6 weeks of storage period (SP) at 13°C. Vertical bars represent LSD ($P \leq 0.05$). LSD ($P \leq 0.05$): T = ns, SP = 0.49, T x SP = ns. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, MJ = methyl jasmonate. ns = not significant.

5.4. Discussion

5.4.1. Fruit texture and softening enzymes

The data showed that combination of MJ treatment with CA storage maintained firmness better than MJ treatment alone but it was not better than CA storage alone. Previous studies on mango revealed that MJ treatment did not affect the firmness of mango cv. 'Tommy Atkins' stored under low temperature (Gonzalez-Aguilar et al., 2000). In other crops, MJ treatment reduced the fruit firmness such as strawberry (Perez et al., 1997) and papaya in MAP (Gonzalez-Aguilar et al., 2003), but reduced the softening of tomato stored in MAP (Siripatrawan and Assatarakul, 2009). MJ, the volatile ester of JA, induces C_2H_4 emission in climacteric fruit (Fan et al., 1998a; Peña-Cortés et al., 2005; Saniewski and Czapski, 1983) including mango fruit cv. 'Kensington Pride' (Lalel et al., 2003g) which may have induced the ripening process suggested by higher fruit softening during storage of MJ-treated fruit regardless of the atmosphere compositions (Table 5.1). It may also be argued that the response of plant to MJ and atmosphere composition during storage is phenotypically dependent.

CA storage and its combination with MJ treatment also affected springiness and adhesive force, but not cohesiveness, gumminess, and fracture force of mango fruit (Table 5.1). However, the duration of storage significantly affected texture profiles except for the fracture force. Springiness is defined as the distance between the end of the first compression and the start of the second compression (Al-Haq and Sugiyama, 2004; Banjongsinsiri et al., 2004). Although the springiness of all treatments significantly decreased during storage, mango stored in CA was the most durable in retaining its shape after the compression. The ability of CA storage to withstand the ripening process was also exhibited by the capability of the fruit treated with MJ and stored in CA which regained the shape better than control and MJ alone. The result suggested that the function of MJ as the ripening regulator (Kondo et al., 2004b) was maintained during CA storage. Adhesive force consists of the negative force area for the first compression (Banjongsinsiri et al., 2004), and represents the work required to overcome the attractive forces at the end of the first compression cycle between the surface of the sample and the surface of the probe (Al-Haq and Sugiyama, 2004; Banjongsinsiri et al., 2004). The fruit treated with MJ and stored in CA exhibited the highest adhesive force compared to other treatment. This result was similar to that reported by Banjongsinsiri (2004) in 'Kent' mango fruit treated with calcium chloride.

PG and PE are pectolytic enzymes responsible for the cell wall degradation during ripening of mango fruit (Abu-Sarra and Abu-Goukh, 1992; Ali et al., 2004). PG hydrolyses the α-(1-4)-linkage of galacturonic acid chain (Lazan et al., 1993) and is classified in two forms, *exo*- and *endo*-PG which are basically differentiated by their specific site of cleavage on substrate (Abu-Sarra and Abu-Goukh, 1992; Lazan et al., 1993; Suntornwat et al., 2000). The activity of *exo*-PG, that cleaves at the terminal end of the polygalacturonic acid chain (Abu-Sarra and Abu-Goukh, 1992; Lazan et al., 1986; Prasanna et al., 2007) hence frees galacturonic acid, was induced at four weeks before declining at 6 weeks storage regardless of the storage condition and treatments, although the alteration of *exo*-PG activity was in different levels (Figure 5.2). The effect of CA storage was stronger than other treatments in triggering the *exo*-PG activity at 4 weeks storage whilst MJ may have lessened the CA effect (Figure 5.2) when MJ-treated fruit stored in CA. Argenta et al. (2010) reported that MJ treatment minimised the level of CO_2 injury in apple fruit, which

may affect 'Kensington Pride' mango similarly. Conversely, *endo*-PG activity that cleaves to glycosidic bonds randomly (Abu-Sarra and Abu-Goukh, 1992; Lazan et al., 1993; Lazan et al., 1986; Suntornwat et al., 2000) was depressed under CA storage and CA storage combined with MJ treatments within two weeks storage while that under control and MJ treatment alone was depressed at six weeks storage (Table 5.2). The activity of PE, the enzyme responsible for de-esterification of methyl group in galacturonic acid (Abu-Sarra and Abu-Goukh, 1992), was lower on week 2 during storage and remained steady until six weeks storage of 'Kensington Pride' mangoes in all treatments. It was reported that low temperature increases the *exo*-PG activity but reduce the *endo*-PG and PE activities in peach (Meng et al., 2009). This phenomenon of pectolytic enzyme activities is demonstrating the effect of CA that depressed the *endo*-PG and PE enzymes activity and diminished the nature of MJ and further reduced the fruit softening (Batu and Thompson, 1998; Lalel et al., 2005) associated with fruit ripening (Noomhorm and Tiasuwan, 1995). Fruit firmness was highly correlated to *endo*-PG and PE, but not *exo*-PG activites (Table 5.3). Fruit firmness was reduced significantly at 2 weeks storage (Table 5.1) as the *endo*-PG and PE activity were depressed. *Exo*-PG activity increased after 4 weeks storage which may be the starting point of ripening process. Increasing activity of *exo*-PG has been reported in 'Nam Dok Mai' mango as the ripening progressed (Chaimanee et al., 2000), however the storage temperature and MJ treatment may have influenced the activity of this enzyme hence the activity was depleted at 6 weeks of storage.

5.4.2. Fruit skin colour and pigments

CA, either by it self or in combination with MJ treatment, was involved in maintaining the chromaticity of fruit skin, including L*, a*, b*, C* and hue° (Figure 5.2), whilst control fruit and MJ-treated fruit had significantly improved skin colour within 6 weeks in storage. The visual observation on 'Kensington Pride' mango skin also resulted in similar trend as chromaticity values monitored through spectrophotometer (Figure 5.2). The inhibition of skin colour development due to CA storage has been reported on 'Delta R2E2' mango fruit by Lalel et al. (2005). Similar result also has been reported when MJ-treated papaya fruit was packed under modified atmosphere (Gonzalez-Aguilar et al., 2003). However, JA alone was reported to improve apple (Fan et al., 1998a) and mango skin colour with high L*

value (Tasneem et al., 2004). Colour development in MJ-treated fruit may be due to the increasing C_2H_4 emission as has been reported in 'Delta R2E2' mango by Lalel et al. (2003g), since MJ stimulates the ethylene-forming enzyme (Czapski and Saniewski, 1992).

Chlorophylls concentration in skin of MJ-treated and control mango fruit decreased on week 2 in storage, whereas MJ-treated fruit lost more than control fruit. MJ treatment is known to cause leaf chlorosis, and senescence-like symptoms (Creelman and Mullet, 1997). The loss of chlorophyll in MJ-treated tomato is reported to be associated with the increasing ethylene-forming enzyme (Saniewski et al., 1987) which may have occurred in MJ-treated mango. Reduction in chlorophylls on CA-stored fruit and MJ-treated fruit combined with CA storage was also recorded but those mangoes retained higher chlorophylls throughout the storage period compared to control and MJ-treated fruit (Figure 5.3). A delay in chlorophylls degradation during CA storage was expected since this effect of CA storage has been reported by many researchers (Kader, 2002; Lalel et al., 2001; Lalel et al., 2005; McLauchlan and Barker, 1992). Accompanied by delayed chlorophylls degradation, carotenoids synthesis was also retarded during CA storage. On 4th week of storage, the carotenoids content was depleted in CA-treated fruit whilst at the same time, that was peaked in MJ-treated fruit (Figure 5.3). MJ promotes β-carotene biosynthesis in mango, tomato, banana, guava (Saniewski et al., 2010) and apple (Perez et al., 1993; Saniewski et al., 2010) which could be related directly or indirectly to C_2H_4 production (Perez et al., 1993). Their combination effect may have been depicted in the delayed chlorophylls degradation and preserved the carotenoinds content during this study on MJ-treated fruit stored under CA condition.

5.4.3. Sugars and organic acids

Fructose and sucrose were the major sugars in green mature 'Kensington Pride' mango, while glucose had an almost undetectable amount (Figure 5.4). Total sugars in mango pulp increased significantly in all treatments within two weeks of storage, with MJ-treated and CA-treated fruit accumulated the highest and the lowest contents of total sugars, respectively. MJ-treated fruit stored under CA condition accumulated sugars throughout six weeks storage period with sucrose as the predominant sugar followed by fructose and glucose. Increase in sugars content is a ripening incidence in fruit where starch degrades into simple sugars while sucrose is

synthesized (Castrillo et al., 1992; Fuchs et al., 1980; Mattoo and Modi, 1969; Tandon and Kalra, 1984). It was reported that sugar content in mango fruit (cv. 'Alphonso' and 'Banganapali') increased during four weeks in CA storage (3% O_2 and 5% CO_2) which indicates the initiation of ripening (Rao and Rao, 2008). Total sugars in CA-stored fruit remained stable at four weeks storage and increased at the end of the storage period at which 'Kensington Pride' mango may have begin to ripen at this prolonged CA storage. The O_2 composition (3%) in this research may play an important role in carbohydrate metabolism such as TCA cycle and pentose phosphate pathway (Kays, 1991) where sucrose, fructose and glucose were degraded and/or synthesized. MJ-treated and control fruit showed a decrease in their total sugars content at six weeks storage which may be due to the decreasing concentration in sucrose. It was reported that glucose and fructose contents is lower than sucrose concentration in ripe mango fruit (Castrillo et al., 1992). The ratio of sucrose and fructose in mature green 'Kensington Pride' mango fruit prior to storage was 47 : 52. After two weeks in storage, sucrose was synthesized almost twice the initial amount in most treatments, except in MJ treatment combined with CA storage. At six weeks storage, the proportion of sucrose and fructose was almost equal in control and CA-treated fruit but this was not the composition in fruit treated with MJ alone or combined with CA storage. Lower sucrose than fructose (37 : 53) concentration in MJ-treated fruit suggested that MJ treatment seemed to alter the synthesis of sucrose while that effect was diminished by CA storage after six weeks storage, resulted in higher sucrose than fructose (56 : 37) concentration. Pre-storage treatment with MJ and CA storage may have altered differently the degree of carbohydrate metabolism in mango fruit. High sucrose in 'Kent' mango without any changes in its monosaccharides when treated with 10^{-4} M MJ, but low fructose and glucose with no change in sucrose level when treated with 10^{-5} M MJ (Gonzalez-Aguilar et al., 2001). High fructose and glucose in MJ-treated 'Zucchini' squash decreased during storage is reported by Wang and Buta (1999). Guava treated with MJ, regardless of its dose, resulted in high accumulation rate of sucrose, glucose, and fructose has been reported (González-Aguilar et al., 2004). It has been suggested that cultivars and the extension of sugars metabolism are factors that influence the proportion of sugars (Singh and Singh, 2012).

Significant reduction of citric acid (90%) occurred in MJ-treated fruit stored for 2 weeks regardless of storage atmosphere, and no further changes within 6 weeks of storage. Similarly, succinic and malic acids were also reduced at the second week of storage and no further changes afterwards, which affected the total acids of those MJ-treated fruit (Figure 5.5). The effect of MJ treatment in this study was similar to the previous report that organic acids concentrations decreased during storage as an indication of ripening process (Eskin, 1990). In contrast, CA storage increased malic acid concentration at four weeks of storage duration and retained it to the end of storage period, whilst no significant changes of citric and succinic acid content hence resulting in high total acids at the retrieval of fruit after six weeks of storage. The effect of CA storage reported in this study was similar to the previous study on coated 'Tommy Atkins' mango stored for 45 days (Madeiros et al., 2012) and other cultivars under CA storage (Kim et al., 2007; Rao and Rao, 2008). This may be argued that limited O_2 in CA storage reduced the degradation of organic acids used for respiration (Bender et al., 2000c), slowed down the respiration itself (Madeiros et al., 2012), and thus prolonged the storage life (Lalel et al., 2005). Baqui et al. (1974) reported that the activity of TCA enzymes responsible for the degradation of malic, and succinic acids, and synthesis of citric acid increased at the beginning and during maturation of 'Alphonso' mango, respectively. It is also reported that activities of citrate synthase declines during ripening (Medlicott and Thompson, 1985) while malic enzyme declines at post climacteric (Dubery et al., 1984), hence reduction on citric acid is responsible for low acidity in ripe fruit (Medlicott and Thompson, 1985). Degradation of citric acid in 'Irwin' mango stored for 20 days 5°C has been reported by Shivashankara et al. (2004), regardless the fruit maturity levels. Results in this study showed that a significant decrease is not only citric, but also malic and succinic acids levels in fruit treated with MJ alone and that combined with CA storage. Strong influence of MJ to compensate CA storage effects may have also reflected the ability of MJ on responding the low O_2/high CO_2 stresses besides its ability to counteract on low temperature, as reported in previous studies (Gonzalez-Aguilar et al., 2001; Gonzalez-Aguilar et al., 2004). The effect of MJ on the activation or deactivation of TCA enzymes is suggested for further study.

5.4.4. Carotenoids, ascorbic acid and total antioxidants in mango pulp

MJ treatment combined with normal atmosphere storage enhanced the accumulation of carotenoids in 'Kensington Pride' mango pulp, which similar to that reported by Saniewski et al. (2010). In contrast, the accumulation of carotenoids in MJ-treated combined with CA storage was suppressed during six weeks storage, and it was not significantly different from fruit stored in CA without MJ treatment (Figure 5.6, Table 5.4). Carotenoids content in 'Alphonso' and 'Banganapalli' mangoes is improved during CA storage (Rao and Rao, 2008). Lalel et al. (2001) reported that carotenoids content of CA-stored 'Kensington Pride' mango increases with increasing CO_2 concentration whilst in my research finding carotenoids content in CA-treated fruit was significantly retarded. The CO_2 concentration (5%) may be the factor that retards the carotenoids accumulation during storage, and this may be similar to the carotenoids synthesis in MJ-treated fruit with concomitant CA storage. The CO_2 concentration may have veiled the effect of MJ on improving carotenoids concentrations.

Ascorbic acid content reduced significantly at week two in MJ-treated fruit and MJ-treated fruit with CA storage compared to the other two treatments, whereas it reduced to its lowest concentration in CA-stored fruit without MJ treatment at week four which expelled similar trend to control fruit (Figure 5.7, Table 5.4). The effect of MJ combined with CA strorage on ascorbic acid content in mango fruit has not been previously reported to the best of my knowledge. Increasing ascorbic acid concentration in 'Alphonso' mango due to low temperature storage has been reported by Thomas (1975), whereas a decreasing effect of high CO_2 on ascorbic acid in berry fruit has been reported by Agar et al. (1997). It has been reported that MJ treatment does not affect the ascorbic acid level concentration in guava fruit (Gonzalez-Aguilar et al., 2004) but improve in peach (Jin et al., 2009).

Total antioxidant activity was significantly affected by storage period but not by MJ treatment and/or CA storage (Figure 5.8, Table 5.4). During 6 weeks storage, the total antioxidant of MJ-treated fruit decreased significantly at 4 and 6 weeks of storage, whereas there was little change in fruit from the other treatments, including MJ-treated fruit in CA. This result does not agree with previous studies. Increasing antioxidant activity due to MJ treatment has been reported in mango (Wang et al., 2006), loquat (Cao et al., 2009a), and pomegranate (Sayyari et al., 2011). Different

phenotype of fruit may be contributed to this variation, and CA storage may be controlled the degradation level of total antioxidant activity in mango pulp.

In conclusion, MJ treatment together with CA storage reduced firmness, *exo*-PG activity and total organic acids, preserved skin chlorophylls and green colour, while improved total sugars in 'Kensington Pride' mango fruit stored under CA condition within 4 weeks at 13 ± 0.5°C. However, MJ treatment combined with CA storage did not improve carotenoids, ascorbic acids, and total antioxidants levels in fruit pulp.

Chapter 6

Controlled atmosphere storage of 'Kensington Pride' mango (*Mangifera indica* L.) pre-treated with putrescine

Summary

Mango fruit are highly perishable leading to rapid deterioration in quality. The objective of this study was to evaluate the effect of pre-controlled atmosphere storage application of putrescine (Put) on firmness, fruit softening enzyme kinetics, and other quality changes in 'Kensington Pride' mango. Green mature 'Kensington Pride' mangoes were dipped in 10^{-3} M Put and 0.01% Tween20 for 6 minutes, air-dried and stored in ambient atmosphere or in CA chambers containing recommended levels of 3% O_2 and 5% CO_2 at 13 ± 0.5°C and 85 ± 3% RH. The fruit were transferred after 2, 4, and 6 weeks of CA storage to ambient conditions. The assessments were made for fruit texture, pectolytic enzymes activities, skin colour and pigments, pulp sugars and organic acids concentration. The overall quality of the Put-treated fruit without CA storage was not better than untreated fruit. However, pre-CA storage application of Put maintained fruit firmness and springiness, increased *exo*-Polygalacturonase (*exo*-PG), reduced *endo*-polygalacturonase (*endo*-PG) and pectin esterase (PE) activity, retarded chromaticity changes, chlorophylls degradation and carotenoids formation in skin, increased accumulation of sugars and organic acids, and maintained carotenoids, ascorbic acid, and total antioxidants in pulp. In conclusion, Put treated fruit followed by CA storage retained fruit firmness and springiness, which is negatively correlated to the *exo*-PG enzyme activity, retarded colour devlopment, increased sugars and organic acids accumulation within 4 – 6 weeks of storage.

6.1.Introduction

Mango (*Mangifera indica* L.) is a climacteric tropical fruit having short shelf life due to its perishable nature and sensitivity to storage temperature. The distance of transportation and marketing of mango fruit were limited due to the vast changes

in biochemical and physiological after harvest (Singh and Singh, 2012). Various techniques have been proposed to prolong the storage life of this delicate fruit, and among them is controlled atmosphere (CA) storage combined with low temperature. CA storage has been reported to affect quality performances of the Australian leading mango cultivar 'Kensington Pride' (Lalel et al., 2001; Lalel et al., 2004), although different CA composition seems to influence the fruit quality at different levels (Singh et al., 2013; Singh and Singh, 2012).

CA storage reduces the C_2H_4 production rate in mango fruit (Lalel et al., 2005) hence prolongs the storage life for long distance trading (Singh et al., 2013). Besides its effect on C_2H_4 and respiration, CA storage has also reported to delay chlorophyll degradation (Lalel et al., 2005; Rao and Rao, 2008) by reducing enzyme activity, and increase sugars content (Rao and Rao, 2008) in mangoes. Prolonging storage life of fresh horticultural produce coupled with maintainance the fresh quality, preserving the bioactive compounds and antioxidant activity without compromising the safety, appearance or sensory properties to meet the consumer's demand (Serrano and Diaz-Mula, 2011).

Polyamines (PA), a class of plant growth regulators, is involved in modulating a range of biological processes including fruit ripening, senescence, production of secondary metabolites, activity of enzymes and responses to stress (Bouchereau et al., 1999; Fariduddin et al., 2013; Kussano et al., 2008; Malik, 2003). PAs share the same precursor, S-adenosylmethionine (SAM), as C_2H_4 in their synthesis. However simultaneous accumulation of PAs and C_2H_4 during ripening depicts their biosynthesis as a causative effect which has been reported in mango fruit (Malik and Sing, 2004). PAs have antisenescence activity; however their concentration in fruit usually decreases during ripening, hence reducing fruit firmness and storability (Kumar et al., 1997). Exogenous application of PAs, either spermidine (Spd), spermine (Spm), or putrescine (Put), have shown some advantageous effects in improving textural attributes and shelf life in some climacteric and non-climacteric fruit including mango, plum, apricot, and strawberry (Khosroshahi et al., 2007; Malik and Singh, 2005; Martínez-Romero et al., 2002; Perez-Vicente et al., 2002). The application of PAs delayed fruit colour degradation (Martínez-Romero et al., 2002; Martínez-Romero et al., 2001; Mirdehghan et al., 2007), increase carotenoids and sugars while reduce acids concentration (Malik and

Singh, 2006) and softening, including reduction in fruit softening enzymes activity (Khan and Singh, 2007b; Razzaq et al., 2014; Torrigiani et al., 2008) during storage while maintained fruit firmness at ripe stage without a significant diminution in ethylene synthesis during ripening. Singh et al. (2013) suggested an inverse relationship between Put concentration and C_2H_4 production.

The exogenous application of PAs on fruit is to enrich their concentration for antisenescence outcomes as the levels of PAs decline with the progression of ripening and senescence. Accumulation of PAs has been reported in cucumber sealed in modified atmosphere packaging (MAP) that improved the chilling tolerance better than control fruit (Wang and Qi, 1997). Undesirable changes during storage of pomegranate fruit were significantly diminished by combined application of Put and carnauba wax treatment (Barman et al., 2011). To the best of my knowledge, the effects of PAs combined with CA storage in prolonging storage life and maintaining quality performance of 'Kensington Pride' mango have not been investigated. It was hypothesized that a combination of Put and CA storage might be more effective in improving storage stability of mango fruit than either of them alone. The objective of this study was to evaluate the effect of pre-CA storage application of Put on firmness, softening enzyme activities, and other quality changes including colour, pigments, carbohydrate and acids compositions, and nutritional composition of 'Kensington Pride' mango.

6.2. Materials and methods

6.2.1. Fruit and experimental conditions

Green mature 'Kensington Pride' mangoes were sourced from a commercial orchard located at Chittering (long. 116°5'E, lat. 31°25'S), Western Australia in March 2007. Fruit were treated and transported to the Curtin Horticultural Research Laboratory in Perth, Western Australia as described in section 3.1. Uniformly mature fruit, free from visual symptoms of any diseases or blemishes were chosen for the experiment.

The experiment was designed by following a completely randomised design. Ten fruit were used as treatment unit and replicated 3 times. Each lot was dipped in a solution contained 10^{-3} M putrescine and 0.01% Tween20 for 6 minutes. The fruit were air-dried and stored in normal atmosphere (NA) and in 90-L chambers of

controlled atmosphere (CA) containing 3% O_2 and 5% CO_2 at 13 ± 0.5°C and 85 ± 3% RH. Concentration of O_2 and CO_2 in the CA chambers were adjusted with N_2. The CA storage was a continuous gas flow with open ended system, and the conditions were maintained and monitored as described in section 4.2.1.1. The fruit were removed from CA chamber after 2, 4, and 6 weeks of storage.

6.2.2. Measurement of fruit texture profile

Fruit were cut at the equatorial area of the fruit (2 x 2 cm^2 in width x height) and the texture profiles were analysed by texture analyser as previously described in section 3.3.2.2.

6.2.3. Fruit softening enzyme activity analysis

The activity of fruit pulp softening enzymes (*exo*-polygalacturonase (*exo*-PG), *endo*-polygalacturonase (*endo*-PG), and pectin esterase (PE)) activities were determined as detailed in section 3.4.

6.2.3.1. Extraction and determination of pectolytic enzymes activity

The extraction and determination of *exo*- and *endo*-PG, and PE activities, from pulp tissues were conducted as detailed in section 3.4.1 and 3.4.2.

6.2.3.2. Protein analysis

Determination of protein content from pulp tissue was performed following the method described in section 3.4.3.

6.2.4. Fruit colour assessment

Visual and Hunter scale measurements were used in assessing the fruit skin colour. Individual fruit from each replication was evaluated during CA storage of control and Put-treated samples. The subjective method the objective skin colour was recorded as detailed in section 3.5.1 and 3.5.2.

6.2.5. Skin pigment analysis

Skin pigments were determined as described in section 3.6.

6.2.6. Sugars and organic acids analysis

High performance liquid chromatography (HPLC) was used in sugars and organic acids determinations. Sugars and organic acids were extracted from mango

pulp tissues and calculated against the external standards as detailed in section 3.7.1 and 3.7.2.

6.2.7. Pulp total carotenoids analysis

Mango pulp total carotenoids were estimated as described in section 3.8.

6.2.8. Ascorbic acid analysis

The concentration of fruit pulp ascorbic acid was determined as described in section 3.9.

6.2.9. Determination of total antioxidants

Total antioxidant activity was determined following the DPPH method as described in section 3.10.

6.2.10. Statistical analysis

Effects of the Put treatement combined with CA storage on fruit softening, colour changes, and nutritive value of 'Kensington Pride' mango were assessed as detailed in section 3.11.

6.3. Results

6.3.1. Texture profile during storage

Firmness and adhesive force of mango pulp was significantly ($P \leq 0.05$) affected by the treatment and storage duration. Other texture parameters such as cohesiveness, springiness and gumminess were significantly affected ($P \leq 0.05$) by the storage period (Table 6.1). Fruit treated with or without Put and stored in normal atmosphere at 13 ± 0.5°C and 85 ± 3% RH for 2 weeks lost 93 to 94% of their initial firmness but those stored in CA lost only about 83%. Firmness of fruit under CA, with or without applied Put, was higher compared to NA treatments at week 2, 4, and 6. At four weeks, Put-treated fruit and stored in CA were slightly but significantly firmer than CA alone fruit, suggesting some effect of Put in maintaining firmness. However, across all storage times, there appeared to be little effect of Put treatment on firmness compared to a strong effect of CA. Extension of CA storage resulted in higher firmness than control fruit and Put treatment alone, although they are not significantly ($P \leq 0.05$) different after six weeks in storage. The interaction between

treatment and storage period significantly ($P \leq 0.05$) affected the firmness of mango fruit pulp.

Other texture attributes such as cohesiveness, springiness, gumminess, and adhesive force decreased as the storage duration extended to six weeks, but the effect of CA with or without Put treatment was identified on adhesive force where their mean values was higher ($P \leq 0.05$) compared to other treatments (Table 6.1). The Put treatment, CA, and duration of storage did not affect mango pulp fracture force at $P \leq 0.05$.

6.3.2. Fruit softening enzyme activity during storage

6.3.2.1. *Exo*- and *endo*-polygalacturonase enzyme activities

The activity of e*xo*-PG in the fruit pulp increased significantly ($P \leq 0.05$) and peaked after four weeks storage (Table 6.2). At this time, the *exo*-PG activity of Put-treated fruit stored in CA condition was 46% higher than control fruit and 21% higher than Put-treated fruit stored in NA. The activity of this enzyme in Put-treated fruit was not significantly ($P \leq 0.05$) different from control fruit at 4 weeks storage. In comparison to the initial activity of *exo*-PG in mango fruit, the highest increase after four weeks storage was measured in fruit treated with Put and stored under CA, followed by CA-stored fruit, Put-treated fruit, and NA-stored fruit which were 212%, 172%, 114%, and 67%, respectively. The activity of e*xo*-PG decreased at 6 weeks storage in all fruit, but Put-treated fruit stored in CA showed significantly higher activity compared to control fruit (Figure 6.3A).

Endo-PG activity was significantly ($P \leq 0.05$) affected by storage period but not significantly different among means of treatments (Table 6.2). *Endo*-PG activity of mango fruit decreased significantly ($P \leq 0.05$) during 2 weeks in storage. This enzyme activity in Put-treated and CA-stored was significantly lower than control fruit. The *endo*-PG activity reduced by 81% and 61% in Put-treated fruit stored in CA and NA, respectively (Figure 6.1A). The changes in *endo*-PG activity were not significant until 6 weeks in storage, except that in Put-treated fruit stored in CA which increased 8-fold and 10-fold of its activity during storage at week 2 and 4, respectively.

Table 6.1. Effects of postharvest application of putrescine on texture profile of 'Kensington Pride' mango fruit during 6 weeks of storage at 13°C

Treatments	Storage period (weeks)				*Means*	LSD
	0	2	4	6	(T)	($P \leq 0.05$)
			Firmness (N)			
Control	124.21a	7.87bB	5.18cC	4.83c	*35.52*B	T = 0.69
CA	124.21a	21.49bA	8.14cB	5.49d	*39.83*A	SP = 0.69
Put + NA	124.21a	9.26bB	5.11cC	4.90c	*35.87*B	T x SP = 1.38
Put + CA	124.21a	20.75bA	9.21cA	5.50d	*39.92*A	
Means (SP)	*124.21*a	*14.84*b	*6.91*c	*5.18*d	*37.78*	
			Cohesiveness			
Control	0.06a	0.03bB	0.05ab	0.04b	*0.04*	T = ns
CA	0.06a	0.07aA	0.05a	0.03b	*0.05*	SP = 0.01
Put + NA	0.06ab	0.07aA	0.05b	0.03c	*0.05*	T x SP = ns
Put + CA	0.06a	0.06aA	0.04ab	0.03b	*0.05*	
Means (SP)	*0.06*a	*0.06*a	*0.05*a	*0.03*b	*0.05*	
			Springiness (N)			
Control	3.01a	1.28bC	1.58bB	1.20b	*1.77*	T = ns
CA	3.01a	2.41bA	2.10bAB	1.12c	*2.16*	SP = 0.22
Put + NA	3.01a	2.13bB	1.63cAB	1.21c	*2.00*	T x SP = ns
Put + CA	3.01a	2.33bAB	2.12bA	1.14c	*2.15*	
Means (SP)	*3.01*a	*2.04*b	*1.86*b	*1.17*c	*2.02*	
			Gumminess (N)			
Control	7.33a	0.28bC	0.23bB	0.17b	*2.01*	T = ns
CA	7.33a	1.51bA	0.42bA	0.14b	*2.35*	SP = 0.75
Put + NA	7.33a	0.64bB	0.27bB	0.16b	*2.10*	T x SP = ns
Put + CA	7.33a	1.30bA	0.41bA	0.16b	*2.30*	
Means (SP)	*7.33*a	*0.93*b	*0.33*bc	*0.16*c	*2.19*	
			Fracture force (N)			
Control	1.52a	0.51b	0.76ab	0.71b	*0.88*	T = ns
CA	1.52	5.15	0.57	0.55	*1.95*	SP = ns
Put + NA	1.52	2.12	0.99	1.74	*1.59*	T x SP = ns
Put + CA	1.52	0.68	1.07	0.6	*0.97*	
Means (SP)	*1.52*	*2.12*	*0.85*	*0.9*	*1.35*	
			Adhesive force (N)			
Control	0.62a	0.54aB	0.36bB	0.67aB	*0.55*B	T = 0.09
CA	0.62b	1.02aA	0.57bA	0.81abAB	*0.76*A	SP = 0.09
Put + NA	0.62a	0.61aB	0.35bB	0.60aB	*0.55*B	T x SP = ns
Put + CA	0.62c	1.05aA	0.76bcA	0.97abA	*0.85*A	
Means (SP)	*0.62*a	*0.80*b	*0.51*d	*0.76*c	*0.68*	

n = 30 (10 fruit x 3 replications), ns = not significant at $P \leq 0.05$. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, Put = putrescine, T = treatment, SP = storage period. Values within a row followed by the same lowercase letter(s) are not significantly different and values within a column followed by the same uppercase letter(s) are not significantly different at $P \leq 0.05$.

6.3.2.2.Pectin esterase enzyme activity

Storage period significantly ($P \leq 0.05$) affects PE enzyme activity, however, there were no significant effect of different treatments on its activity (Table 6.2). On

the first 2 weeks in storage, the PE enzyme activity decreased significantly ($P \leq 0.05$). Only about 10% of the initial activities were detected in all fruit treated with or without Put and stored under normal or controlled atmospheres (Figure 6.2). The means activity of pectin esterase of mango fruit pulp increased after 4 weeks in storage, i.e. twice as high as the first 2 weeks.

6.3.3. Correlation analysis of fruit firmness and softening enzymes

There was a significant positive correlation between fruit firmness and *endo*-polygalacturonase and pectin esterase (r = 0.758 and r = 0.948, respectively), as well as significant negative correlation (r = -0.407) between fruit firmness and the activity of *exo*-polygalacturonase (Table 6.3).

Table 6.2. Effects of postharvest application of putrescine on pectolytic enzymes during storage of 'Kensington Pride' mango fruit at 13°C

Treatments	Storage period (weeks)				*Means* (T)	LSD ($P \leq 0.05$)
	0	2	4	6		
			Exo-PG (µg GalA·mg prot^{-1}·h^{-1})			
Control	87.43c	73.32c	146.19aD	112.64bC	*104.90B*	T = 16.47
CA	87.43c	66.40d	237.63aB	127.96bC	*129.86AB*	SP = 16.47
Put + NA	87.43c	70.34d	187.56aC	149.62bB	*123.74AB*	T x SP = 32.93
Put + CA	87.43c	78.95c	272.81aA	171.94bA	*152.78A*	
Means (SP)	*87.43c*	*72.25c*	*211.05a*	*140.54b*	*127.82*	
			Endo-PG (Viscosity changes in sec·mg prot^{-1}·h^{-1})			
Control	15.63a	8.83bA	0.00dB	0.63cB	*6.27*	T = ns
CA	15.63a	0.74cB	1.84bA	0.00dB	*4.55*	SP = 2.83
Put + NA	15.63a	2.97bB	3.12bA	0.68bB	*5.60*	T x SP = ns
Put + CA	15.63a	6.02bA	1.18cAB	13.91aA	*9.19*	
Means (SP)	*15.63a*	*4.64b*	*1.54b*	*3.81b*	*6.40*	
			PE (mM NaOH·mg prot^{-1}·h^{-1})			
Control	0.176a	0.015c	0.039b	0.016c	*0.061*	T = ns
CA	0.176a	0.017c	0.043b	0.019c	*0.064*	SP = 0.018
Put + NA	0.176a	0.018c	0.038b	0.030bc	*0.065*	T x SP = ns
Put + CA	0.176a	0.019d	0.047b	0.030cd	*0.068*	
Means (SP)	*0.176a*	*0.017b*	*0.042b*	*0.024b*	*0.065*	

n = 3 replications, ns = not significant at $P \leq 0.05$. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, Put = putrescine, T = treatment, SP = storage period.

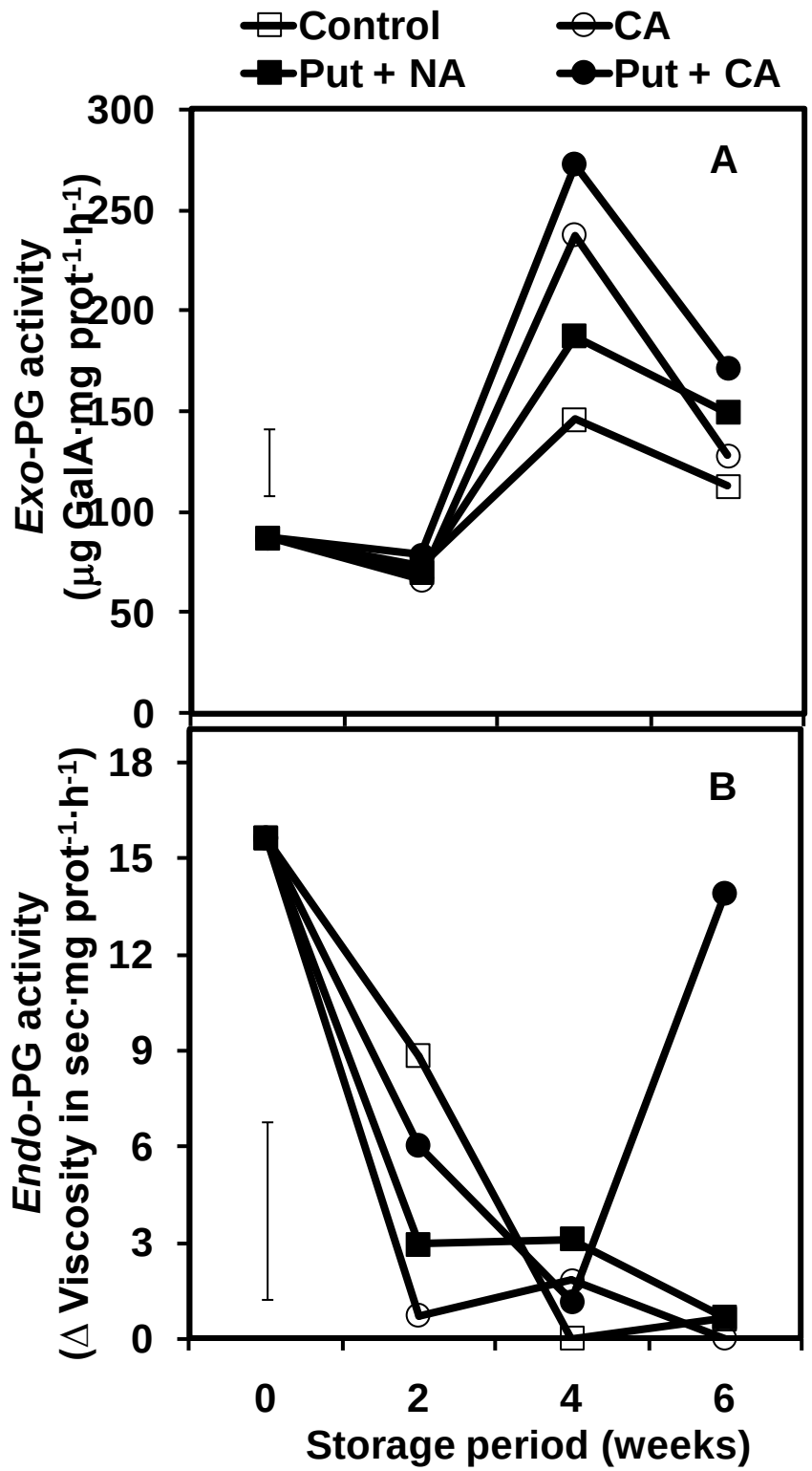

Figure 6.1. (A) *Exo*- and (B) *endo*-PG activity of mango fruit pulp as influenced by Put and CA (T) within 6 weeks of storage (SP) at 13 ± 0.5°C. Vertical bar represents LSD ($P \leq 0.05$), n = 15 (5 fruit x 3 replications). LSD for (A) *exo*-PG: T = 16.47, SP = 16.47, T x SP = 32.93; (B) *endo*-PG: T = ns, SP = 2.83, T x SP = ns. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, Put = putrescine.

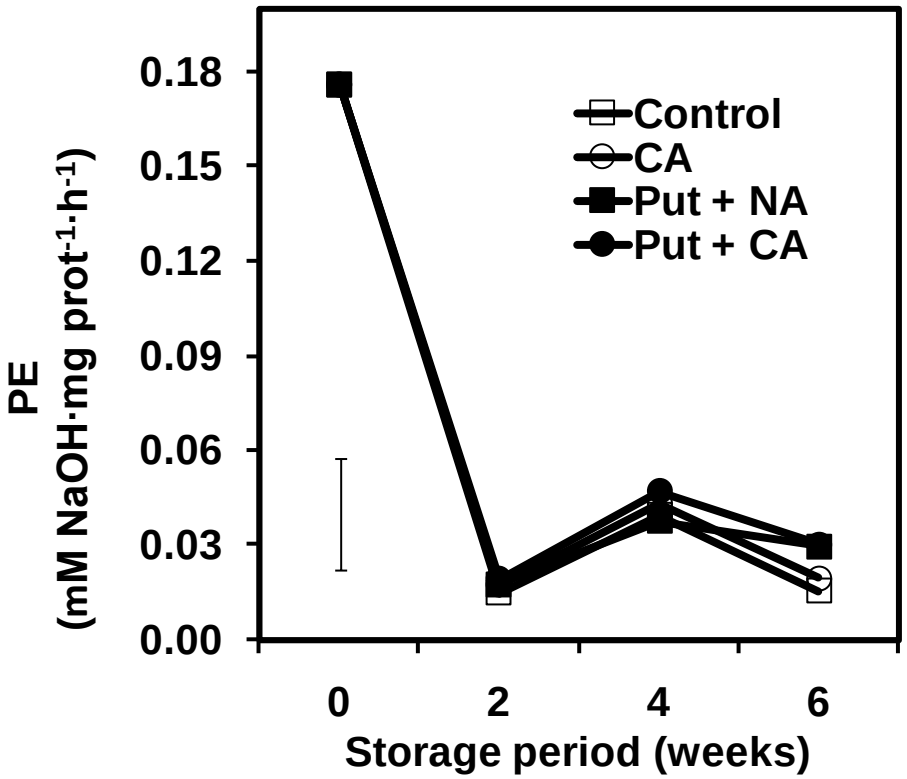

Figure 6.2. Changes in PE activity in the fruit pulp as influenced by Put and CA (T) during 6 weeks of storage (SP) at 13 ± 0.5°C. Vertical bar represents LSD ($P \leq 0.05$), n = 15 (5 fruit x 3 replications). LSD ($P \leq 0.05$): T = ns, SP = 0.018, T x SP = ns. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, Put = putrescine.

Table 6.3. Correlation of fruit firmness and softening enzymes activity in pulp tissues of 'Kensington Pride' mango treated with putrescine during CA storage

Variable compared	Pearson's correlation coefficients (r)
Firmness vs *Exo*-polygalacturonase	-0.407**
Firmness vs *Endo*-polygalacturonase	0.758***
Firmness vs Pectin esterase	0.948***

, * = significant at $P \leq 0.01$ or 0.001 respectively

6.3.4. Fruit skin colour

Fruit skin colour were significantly ($P \leq 0.05$) affected by Put treatment and duration of CA storage. The skin colour lightness (L*), a*, b*, and colour saturation (C*) or chroma of Put-treated and control fruit were significantly higher within six weeks in storage under normal atmosphere condition (Figure 6.3. A, B, C, and D) but exhibited declining hue° (Figure 6.3. E). However, CA-stored mango fruit with or without Put pre-treatment showed no significant changes of L*, a*. b*, C* and hue° (Figure 6.3 A, B, C, D, and E) within six weeks storage. The development of chromaticity of fruit skin was obstructed considerably by CA storage within six weeks, irrespective of the Put treatment. In accordance to the spectrophotometer colour measurement, the visual colour of NA-stored fruit developed more yellow in colour than CA-stored fruit during six weeks of storage, with or without Put treatment, suggesting little effect of Put treatment on mango skin colour as opposed to a strong effect of CA (Figure 6.3F). These CA-stored fruit were scored at 1.33 – 1.44 whilst fruit stored under normal atmosphere were scored at 5.0, which is the highest visual score (100% yellow).

6.3.5. Skin pigments during storage

The concentration of chlorophyll a is higher than b with the initial ratio of 2.9 : 1 and reduced to 2.6 : 1 after six weeks storage with similar trend of reduction. In general, the chlorophyll concentration in mango skin was significantly influenced by treatments and duration of storage. The chlorophyll concentration reduced significantly ($P \leq 0.05$) during 6 weeks storage with lower reduction in CA-stored with or without Put treatment compared to Put-treated and control fruit. During six weeks in CA storage, the fruit significantly retained its chlorophylls by as much as 55%, whereas those stored in normal air significantly lost by 96%, irrespective of the Put treatment (Figure 6.4). Fruit treated with Put combined with CA storage significantly accumulated chlorophylls at four weeks of storage before a drop at week six in storage. In general, the accumulation of chlorophylls was significantly higher in CA-stored fruit than in normal air-stored fruit at the end of storage period.

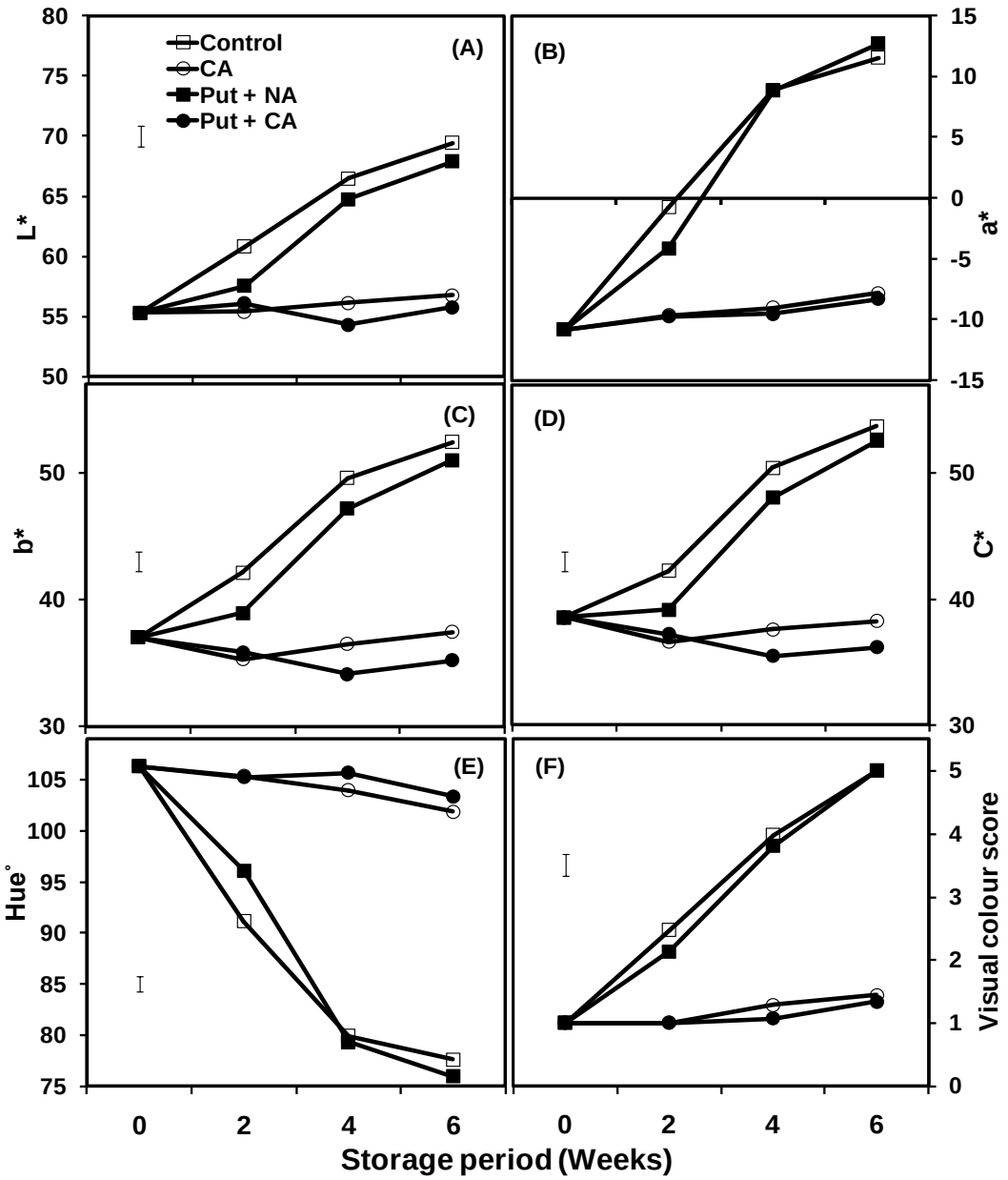

Figure 6.3. Effects of Put and CA (T) on fruit skin colour during 6 weeks of storage period (SP) at 13 ± 0.5°C. Vertical bar represents LSD ($P \leq 0.05$), n = 3 replications. LSD ($P \leq 0.05$) for (A) L*: T = 0.88, SP = 0.88, T x SP = 1.76; (B) a*: T = 0.62, SP = 0.62, T x SP = 1.24; (C) b*: T = 0.80, SP = 0.80, T x SP = 1.60; (D) C*: T = 0.80, SP = 0.80, T x SP = 1.60; (E) Hue°: T = 0.76, SP = 0.76, T x SP = 1.53; (F) visual colour: T = 0.17, SP = 0.17, T x SP = 0.34. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, Put = putrescine.

The concentration of carotenoids in the skin of control fruit increased significantly ($P \leq 0.05$) at week 2 and in Put-treated fruit at week four stored in air at 13°C. The increase in carotenoids content was to the extent of 1.79 and 2.11 fold, respectively (Figure 6.4.). However, the skin carotenoids in those fruit decreased at

the following 1 – 2 weeks after increasing. On the contrary, the levels of carotenoids in fruit stored in CA with or without Put treatment was remained at their initial level until week four and two before declining at week six and four respectively. Carotenoids content reduced in control and CA-stored fruit after four weeks of storage, while in Put-treated stored in air and CA after six weeks of storage. Accounted from the initial level of carotenoids in mango skin, the highest reduction was measured in control, followed by CA-stored, Put-treated with CA storage, and Put-treated fruit, which was 99%, 97%, 96% and 95%, respectively. Put-treated fruit contain higher carotenoids than control fruit after four weeks of storage but it was not significantly different from other treatments after six weeks storage.

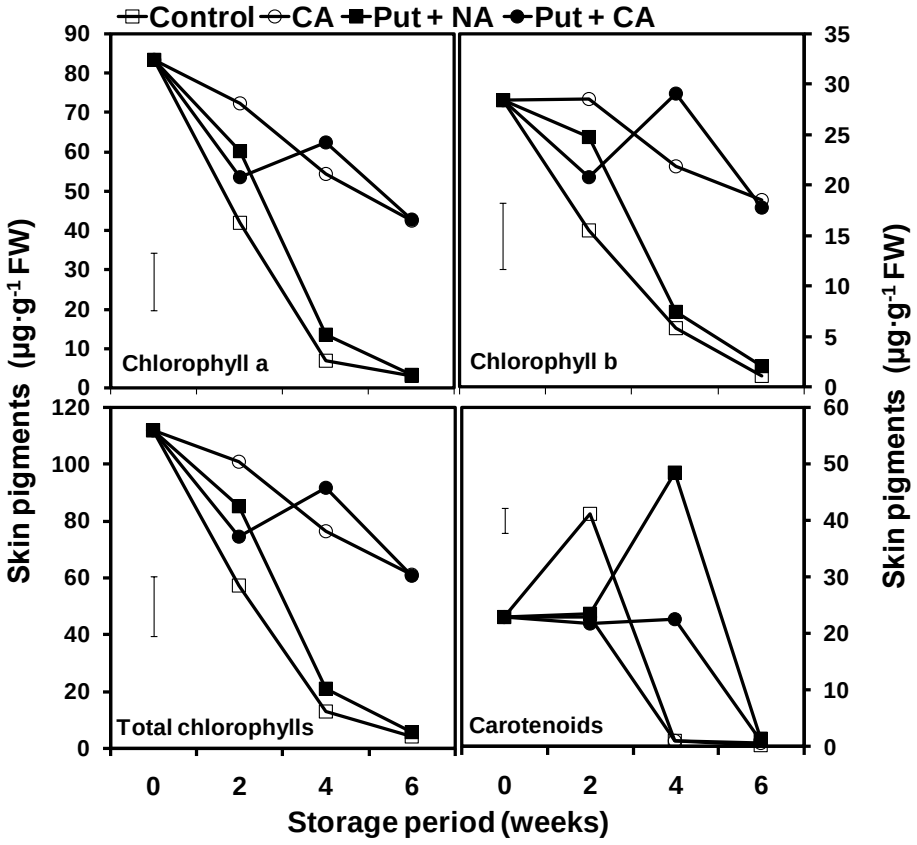

Figure 6.4. Effects of Put and CA (T) on pigments concentration in fruit skin during 6 weeks of storage period (SP) at 13 ± 0.5°C. Vertical bar represents LSD ($P \leq 0.05$), n = 3 replications. LSD ($P \leq 0.05$) for Chlorophyll a: T = 7.38, SP = 7.38, T x SP = 14.76; chlorophyll b: T = 3.28, SP = 3.28, T x SP = 6.56; total chlorophylls: T = 10.64, SP = 10.64, T x SP = 20.92; carotenoids: T = 2.30, SP = 2.30, T x SP = 4.61. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, Put = putrescine.

6.3.6. Sugars in mango pulp during storage

Storage atmosphere and duration significantly affected the sucrose and glucose concentration in mango fruit; and only storage duration had significant effect on fructose and total sugars concentration (Figure 6.5). Sucrose concentration increased during the first 2 weeks in storage with the highest level of increment showed in Put-treated fruit, followed by control, CA-stored, and Put-treated stored in CA, which was 3.4, 3.4, 2.6, and 2.3 fold, respectively without significant difference between control and Put treatment, and between CA-stored fruit with and without Put treatment. Extended storage resulted in reduced sucrose concentration in all fruit but Put-treated fruit stored under CA for six weeks. The sucrose accumulation in Put-treated fruit stored under CA was 1.5 fold higher than the second week of storage when fruit retrieved at the final day of six weeks in storage. The sucrose content in all other fruit was not significantly different after six weeks of storage.

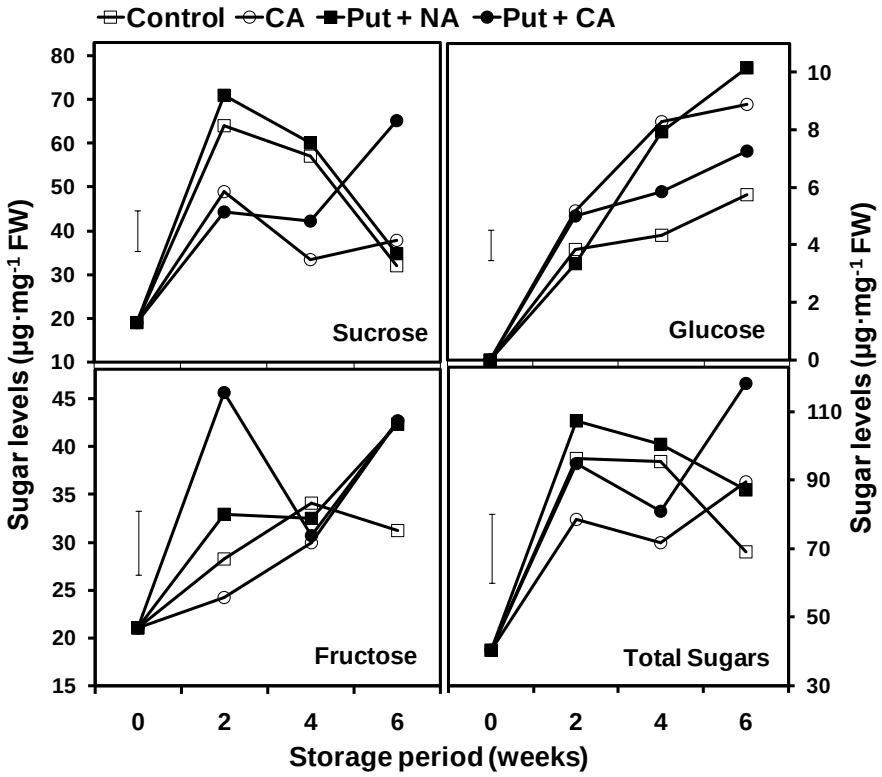

Figure 6.5. Effects of Put and CA (T) on sugar composition in fruit pulp during 6 weeks of storage period (SP) at 13 ± 0.5°C. Vertical bar represents LSD ($P \leq 0.05$), n = 3 replications. LSD ($P \leq 0.05$) for Sucrose: T = 4.58, SP = 4.58, T x SP = 9.16; Glucose: T = 0.52, SP = 0.52, T x SP = 1.05; Fructose: T = ns, SP = 3.34, T x SP = 6.67; Total sugar: T = ns, SP = 10.13, T x SP = ns. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, Put = putrescine.

Glucose concentration increased significantly in the mango pulp within the first 2 weeks of storage. There were no significant changes in glucose content in control fruit within the last 4 weeks of storage. The concentration of glucose in fruit from CA treatment alone was maintained at a similar level within 4 to 6 weeks storage, while it increased significantly in fruit from other treatments. In comparison to control fruit, the accumulation of glucose in Put-treated fruit, CA-treated fruit, and Put-treated fruit stored in CA was 1.3, 1.6, and 1.8 fold higher than control after 6 weeks in storage (Figure 6.5).

The concentration of fructose in Put-treated fruit stored under CA and normal air increased by about 2 and 1.5 fold within the first two week, however only Put treatment combined with CA storages showed significant different with other treatments, including control. Fructose content in Put-treated fruit stored in CA reduced at four weeks before it increased again. After six weeks in storage, the level of fructose accumulation in Put-treated fruit with and without CA storage, and CA-stored fruit was twice of the initial level, and this was significantly higher than fructose content in control fruit.

6.3.7. Organic acids in mango pulp during storage

Treatments and storage period had a significant effect on organic acids concentration in mango pulp (Figure 6.6). Citric acid concentration in control fruit decreased during four weeks of storage. Put treatment was able to maintain its citric acid content during four weeks of storage but not for the extended two weeks afterwards. However, the concentration of citric acid was preserved well in fruit from Put treatment together with CA storage for 6 weeks at 13°C. Put-treated fruit and stored in CA contained significantly ($P \leq 0.05$) higher citric acid, followed by CA-stored and Put-treated fruit compared to control, and it was 24.5, 22.7, and 15.8 fold higher than control which only contained 1.35 $\mu g \cdot mg^{-1}$ citric acid.

Malic acid concentration in control mango pulp increased significantly ($P \leq 0.05$) after four weeks of storage before decreasing after six weeks of storage. Similarly, increasing malic acid concentration was showed in all CA-stored fruit but it remained stable until 6 weeks of storage. However, there was an increase in malic acid content in fruit treated with Put without CA storage after the second to fourth week of storage, and it decreased significantly after six weeks of storage to the initial

level. Within four weeks of storage, the level of increment in malic acid concentration, accounted from its initial concentration, was 65% in both Put-treated fruit with or without CA storage, and 59% in CA-stored fruit whereas 54% in control fruit. The extended storage duration up to 6 weeks markedly affected malic acid accumulation in each treated fruit (Figure 6.6). The combination effect of Put treatment and CA storage resulted in higher malic acid accumulation compared to CA storage and Put treatment alone, and it was to the extent of 1.3 and 3.3 fold. Moreover, CA treatment alone resulted in 2.6 fold higher malic acid concentration than Put treatment after six weeks of storage. A significant decrease in malic acid content was measured in Put-treated fruit after six weeks of storage which was 3.3 fold lower than that peak at four weeks but not significantly different from the initial concentration and control fruit.

Succinic acid concentration in Put-treated fruit decreased significantly within 4 weeks followed by a significant increase at six weeks of storage. However, the combination treatment of Put and CA storage maintained the succinic acid content in fruit pulp until four weeks and subsequently increased it at six weeks of storage. The duration of storage did not affect the concentration of succinic acid in CA-stored fruit, since there were no significant changes throughout six weeks of storage. At the last week of observation, Put-treated fruit combined with CA storage and Put-treated fruit stored in regular air showed significantly higher succinic content compared to CA-stored, and control fruit, and it was about 1.4, and 1,6 fold higher.

In general, total acids content in mango pulp was significantly affected by treatments, storage duration, and their interaction ($P \leq 0.05$). The accumulation of organic acids showed a decreasing trend in control fruit in contrast with an increasing trend in Put-treated fruit, irrespective of its storage atmosphere condition. Total acids content was at its lowest level in Put-treated fruit after four weeks and at its highest after six weeks of storage, whilst the content of total acids remained stable in fruit from CA treatment (Figure 6.6).

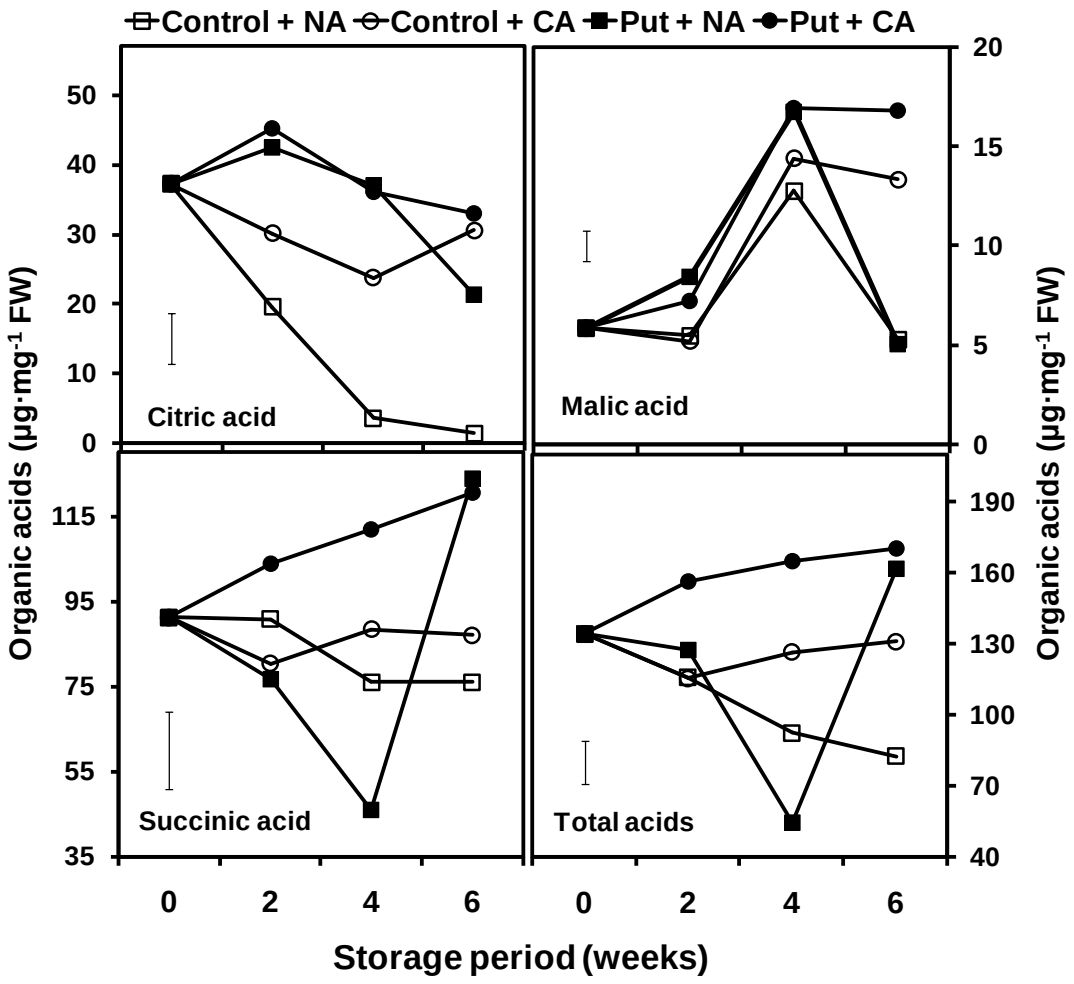

Figure 6.6. Effects of Put and CA (T) on organic acids composition in fruit pulp during 6 weeks of storage period (SP) at 13 ± 0.5°C. Vertical bar represents LSD ($P \leq 0.05$), n = 3 replications. LSD ($P \leq 0.05$) for Citric acid: T = 3.55, SP = 3.69, T x SP = 7.38; Malic acid: T = 0.66, SP = 0.75, T x SP = 1.05; Succinic acid: T = 7.90, SP = 9.10, T x SP = 18.21; Total acids: T = 10.02, SP = 11.63, T x SP = 23.25. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, Put = putrescine.

6.3.8. Carotenoids, ascorbic acid and total antioxidants in mango pulp

Carotenoids levels increased in mango pulp during the first week of storage, irrespective the storage atmosphere and Put treatment. There was another significant increase at four weeks of storage in CA-stored fruit and control, but no further changes afterwards. Level of carotenoids remained constant in fruit treated by Put combined with CA or in air storage between two to six weeks of storage. During six

weeks of storage, the concentration of carotenoids increased by about 22, 5, and 7 fold in Put-treated, Put-treated stored in CA, and CA-stored fruit, respectively; while there was a 16 fold in control fruit. The carotenoids content was significantly higher in fruit with Put treatment compared to control and CA storage alone or in combination with Put treatment after six weeks of storage; however no significant difference was observed in CA-stored fruit, irrespective of Put treatment (Figure 6.7).

The concentrations of ascorbic acid decreased by about 37%, 77%, 72%, and 44% in CA-stored, Put-treated, Put-treated with CA storage, and control fruit, respectively during the first 2 weeks of storage. With the prolonged storage period to four weeks, the concentration of ascorbic acid decreased significantly in all fruit, except those under Put treatment in combination with CA storage (Figure 6.7). From four to six weeks of storage, ascorbic acid concentration decreased in Put-treated fruit stored under CA condition as opposed to other treatments. Mostly, the ascorbic acid remained less than half of the at-harvest concentration. The level of ascorbic acid accumulated after 6 weeks of storage was significantly higher in CA-stored fruit compared to the other treatments.

Total antioxidants levels decreased in all treatments during the first two weeks of storage (Figure 6.7) but only fruit treated with Put without CA storage showed significant reduction during storage. At 4 and 6 weeks of storage, the content of total antioxidants in Put-treated fruit (1.47 μmol TE·100 g^{-1} FW) were significantly lower than those in fruit from the other three treatments (i.e. 3.40, 3.14, and 2.92 μmol TE·100 g^{-1} FW in CA, Put+CA, and control fruit, respectively) with little treatment differences among the later.

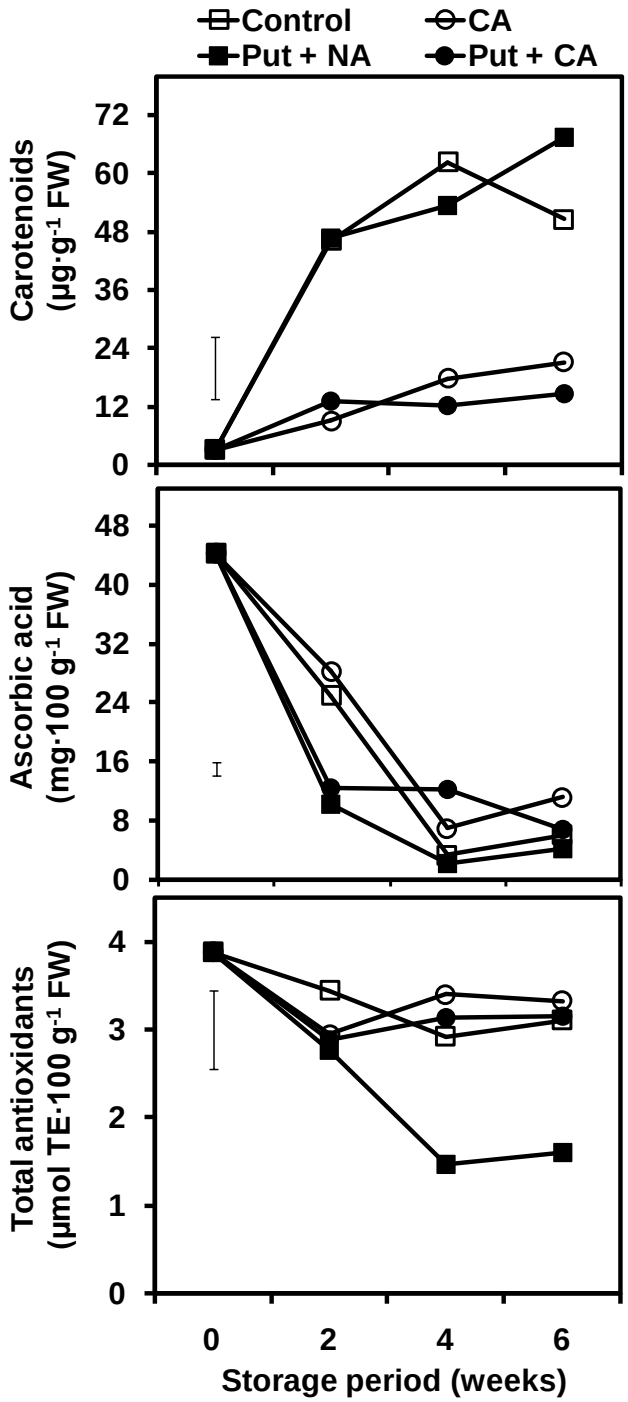

Figure 6.7. Effects of Put and CA (T) on carotenoids, ascorbic acid, and total antioxidants in fruit pulp during 6 weeks of storage period (SP) at 13 ± 0.5°C. Vertical bar represents LSD ($P \leq 0.05$), n = 3 replications. LSD ($P \leq 0.05$) for carotenoids: T = 6.42, SP = 6.48, T x SP = 12.95; ascorbic acid: T = 86, SP = 0.88, T x SP = 1.77; Succinic acid: T = 0.46, SP = 0.45, T x SP = ns. CA = controlled atmosphere (3% O_2 + 5% CO_2), NA = normal atmosphere, Put = putrescine.

6.4. Discussion

6.4.1. Fruit texture and softening enzymes

Alteration in O_2/CO_2 composition suppresses the respiratory system and C_2H_4 production in fruit, resulting in retardation of ripening process (Beaudry, 1999). The delay in fruit softening is an indication in delaying ripening process, which prolongs storage life of fruit. In tomato fruit, the ripening-related softening was inhibited during CA storage after initial loss of firmness (Ingham et al., 1998) which may be a similar response of mango in this research. The superiority of CA storage alone in extending the storage life seemed to be impeded when Put treatment was applied prior to CA storage. After 4 weeks of storage, Put-treated fruit stored under CA were firmer than CA-stored fruit. Putrescine, one of plant polyamines, is known as the plant regulator involves in modulating biological processes including fruit ripening and senescence (Fariduddin et al., 2013; Kussano et al., 2008; Malik, 2003). Put treatment was reported to reduce the mango fruit softening during cold storage (Singh et al., 2013). Khan and Singh (2007b) reported that exogenous application of Put significantly reduced softening in plums after three weeks of storage. The reduction in fruit firmness during storage may be an indication of the ripening process which was retarded when fruit stored under CA storage for two weeks and it may be prolonged to four weeks when Put-treated fruit was stored in CA. However, Put treatment together with CA storage showed no effect in retaining fruit firmness after 6 weeks of storage. The combined treatment of Put and CA storage also had significant effect on springiness after 4 weeks of storage compared to control (Table 6.1), which may be ascribed as the effect of Put treatment that augmented the influence of CA storage, hence improved the ability of mango pulp to regain its shape after compression.

Pectolytic enzymes are responsible for the cell wall degradation during mango fruit ripening (Abu-Sarra and Abu-Goukh, 1992; Ali et al., 2004). The activity of *exo*-PG enzyme is at the terminal end of polygalacturonic acid chain whilst that of *endo*-PG enzyme is at random site (Abu-Sarra and Abu-Goukh, 1992; Suntornwat et al., 2000). After 4 weeks of storage, the *exo*-PG activity in mango pulp increased in all treatments but Put treatment in combination with CA storage seemed to affect the activity of *exo*-PG more than other treatments (Figure 6.1.A). The activity of this enzyme reduced as the storage duration prolonged to 6 weeks,

however the Put treatment combined with CA storage had the higher effect on activating the *exo*-PG enzyme in cutting the terminal end to release galacturonic acid. On the contrary, the *endo*-PG enzyme activity reduced after two weeks and no further noticeable changes in fruit stored under CA without Put treatment, in Put-treated fruit stored in normal air as well as control fruit throughout six weeks storage period. However, there was an unexpected increase on *endo*-PG activity in fruit treated with Put combined with CA storage (Figure 6.1.B). PE activity reduced in all fruit after two weeks of storage and remained stable until six weeks of storage, irrespective of storage type and Put treatment (Table 6.2). PE is a pectolytic enzyme responsible for de-esterification of methyl group in galacturonic acid (Abu-Sarra and Abu-Goukh, 1992). CA storage has been reported to retard fruit softening (McLauchlan and Barker, 1992) which could be related to the reduction in PE activity. The effect of Put treatment in plum fruit in reducing PE, *exo*- and *endo*-PG activity during three weeks of cold storage has been reported by Khan and Singh (2007b). The effect of low temperature storage on increasing *exo*-PG activity but reducing *endo*-PG and PE activities has been reported in peach (Meng et al., 2009). Torrigiani et al. (2008) also reported that exogenous application of Put inhibits the cell wall degrading enzyme activities. The effect of Put treatment on inhibiting senescence is due to its interference on the formation of ethylene-froming enzyme, which is essential to the synthesis of C_2H_4 (Glaston and Sawhney, 1990). However, there were no reports regarding the effects of Put treatment on the activity of softening enzymes in mango fruit. The effects of Put combined with CA storage treatment on the acitivity of fruit softening enzymes during mango fruit ripening warrants to be investigated.

Increasing activity of PG enzymes reduces in fruit firmness (Mitcham and McDonald, 1992) although a weak correlation between them has been reported (Abu-Sarra and Abu-Goukh, 1992). The activity of PE during ripening is either increased or constant (Ali et al., 2004; Prasanna et al., 2003; Roe and Bruemmer, 1981). This suggested that pectolytic enzyme activity may not be the single factor triggering softening in mango pulp. There was a positive correlation between fruit firmness with *endo*-PG and PE activity but negative correlation with *exo*-PG activity (Table 6.3). There was reduction in firmness during two weeks in storage as the result of reduced *endo*-PG and PE activities, although the correlation was stronger

with PE activities. Negative correlation showed that when *exo*-PG activity increased, fruit firmness decreased, but the level of correlation was low, which is in accordance to previous research reported by Abu-Sarra and Abu-Goukh (1992).

6.4.2. Fruit skin colour and pigments

The result suggested that Put had little impact on skin colour development from green to yellow as opposed to CA, which retarded it. The visual evaluation on mango fruit also reflected similar trend to the chromaticity in fruit of different treatment (Figure 6.3). Delay in colour development in 'Kensington Pride' mango due to Put treatment has been reported previously (Malik and Singh, 2006). In contrast, CA storage has been reported to suppress the colour development of 'Delta R2E2' mango (Lalel et al., 2005). Lower Lightness (L*) and the chroma (C*) with higher hue° values were noticed in Put-treated 'Kensington Pride' mango compared to control fruit; which may be an indication of Put effect on retarding colour development during storage while C_2H_4 production was unaffected (Singh et al., 2013). However, there was significant retardation on colour development in mango stored in CA with or without pre-storage treatment of Put. This may be explained by the capacity of CA storage in inhibiting C_2H_4 production, hence delayed the ripening process, which was indicated by the suppression of colour development in 'Kensington Pride' mango.

Chlorophyll concentration in mango skin of Put-treated and control fruit decreased significantly through four weeks of storage duration. In contrast, CA-stored fruit retarded the chlorophylls degradation during six weeks of storage, irrespective of Put treatment. Put is not a ripening inhibitor (Tassoni et al., 2006), but it delays colour development during storage (Singh et al., 2013). In this study, delayed in chlorophylls degradation occurred within two weeks storage of Put-treated mango fruit and it was ineffective during prolonged storage. As has been reported by Lalel et al. (2005), CA storage delayed the chlorophyll degradation in 'Delta R2E2'. Rao and Rao (2008) also reported that the inhibition effect of CA on chlorophyll degradation is due to its effect on reducing the chlorophyll degrading enzyme activity. This may give an explanation when chlorophylls degraded in Put treated fruit during six weeks of storage but when it is combined with CA storage, the chlorophylls was retained. The influence of CA seemed to dominate the

preservation of chlorophylls in 'Kensington Pride' mago fruit during storage compared to Put treatment.

As the chlorophyll degraded, the carotenoid was accumulated in mango skin. Pre-harvest treatment of PAs increased carotenoids content in ripe 'Kensington Pride' has been reported by Malik and Singh (2006). In this research, the postharvest Put-treated fruit exhibited the peak of carotenoids concentration at four weeks whereas control fruit after two weeks of storage, suggested that Put may prolong storage life of 'Kensington Pride' mango. When Put treated fruit stored in CA, the concentration of carotenoids was managed on its initial level until week 4 whilst it was depleted in CA-stored fruit without Put treatment (Figure 6.4). CA storage may retard the carotenoids synthesis through the inhibition of ripening process since the disappearance of chlophylls and the accumulation of carotenoids is an indication of mango fruit ripening (Singh and Singh, 2012). The combination effect of Put and CA in managing the colour and pigments of 'Kensington Pride' mango may prolong its storage life.

6.4.3. Sugars and organic acids in mango pulp

The major sugars in green mature 'Kensington Pride' mango were sucrose and fructose. The composition of sucrose and fructose before storage was almost equal while glucose was found in smaller amounts. Total sugars in all fruit increased at week two, with no further increase from two to four weeks of storage. However, as the storage prolonged to six weeks, the sugar content in Put-treated and control fruit decreased while that in CA-stored fruit alone or combined with Put treatment increased (Figure 6.5.). This result was in accordance with previous work reported by Rao and Rao (2008) that sugars concentration of CA-stored mango increased after four weeks of storage. Different duration time of change in sugar level may be due to cultivar response on CA treatment. At the initial step of mango fruit ripening, starch that accumulates in chloroplast was reported to hydrolyse to simple sugar such as sucrose, fructose and glucose (Kumar et al., 1994; Singh and Singh, 2012). Sucrose, fructose, and glucose concentrations increased in all fruit after two weeks in storage. Sucrose concentration declined after 4 – 6 weeks, while fructose and glucose were either stable or increased in Put-treated fruit and control. This may be due to the degradation of starch to sucrose achieved its maximum after two weeks of storage while degradation of sucrose to its reducing sugars was in progress. The

ripening may have started at the second week in these fruit. Malik and Singh (2006) reported that Put treatment improved ripe fruit quality, including sugars concentration. There was lower sucrose accumulation in all CA-stored fruit, with or without Put treatment, at the second week compared to control, however higher accumulation after six weeks CA storage in Put-treated fruit compared to other treatments. Sucrose and glucose concentrations in mango fruit treated with Put and stored in CA were higher than untreated fruit in CA at six weeks storage. Oxygen is essential in carbohydrate metabolism (Kays and Paull, 2004). Reduced O_2 under CA condition may affect the sugars accumulation in 'Kensington Pride' mango. However, when Put-treated fruit stored under CA condition for 4 – 6 weeks, the fruit may gain its normal metabolism due to increase in the synthesis and/or degradation of sugars and starch. Control fruit contained the smallest amount of sugars while Put-treated fruit stored in CA accumulated the largest. The increasing amount of sugars in CA-stored fruit reached the decreasing amount of Put-treated fruit at six weeks of storage. This may suggest that CA storage and Put treatment affects the sugars accumulation in adverse manner, yet the combination exhibits better results.

In green mature 'Kensington Pride' mango, succinic acid was the principal organic acid followed by citric acid and malic acid (Figure 6.6.). Various organic acids are synthesized through the TCA cycle and they decrease as the storage proceeds (Eskin, 1990). Citric acid concentration reduced significantly in control fruit during four weeks of storage. CA-stored fruit also showed a decreasing trend at the same duration time although it was not significant. However, the citric acid concentration in Put treated fruit with or without CA storage was higher than CA-stored without Put treatment during 4 weeks storage. Malic acid concentration increased at four weeks storage followed by significant decrease in Put-treated and control fruit, little change in all CA-stored fruit when the storage duration was prolonged to six weeks. A considerable reduction of succinic acid was noticed in fruit treated with Put without CA storage at the fourth week which followed by a sharp accumulation to six weeks of storage. Succinic, as the major organic acid, contributed to the largest amount of total acids present in 'Kensington Pride' mango. CA storage seems to prevent degradation of organic acids at 4 to 6 weeks storage, which may be explained as the effect of its O_2/CO_2 composition, in which reduce O_2 is reported to reduce degradation of organic acids (Bender et al., 2000c; Rao and

Rao, 2008). In previous work, Malik and Singh (2006) reported that pre-harvest treatment of Put on 'Kesington Pride' mango reduced the concentration of acids in ripe fruit. Postharvest treatment of Put in this research reduced organic acids levels in comparable levels to control fruit, which was mainly due to reducing succinic acid level within six weeks in storage.

In general, Put-treated fruit stored in CA accumulated higher amounts of sugars than Put-treated fruit stored in NA and CA-stored fruit alone. The accumulation of organic acids in mango pulp was higher in fruit treated with Put and stored under CA condition compared to Put-treated fruit and CA stored fruit without Put treatment. As reported earlier that Put is not a ripening inhibitor but a natural compound that can extend the maturity stage (Tassoni et al., 2006), whereas CA storage is a system with altered O_2/CO_2 composition to inhibit the ripening process. The combination effect of Put and CA storage may contribute to the accumulation of sugars and organic acids in mango pulp.

6.4.4. Carotenoids, ascorbic acid, and total antioxidants in mango pulp

During six weeks of storage, the carotenoids in all CA-stored fruit, irrespective of Put treatment, was maintained at lower level compared to Put-treated and control fruit (Figure 6.7), suggesting that Put treatment had little impact on pulp carotenoids content during storage as opposed to CA conditions, which inhibited its synthesis and accumulation. At 2, 4, and 6 weeks of storage, the carotenoids in all CA stored fruit, irrespective of Put treatment, was maintained at lower level compared to Put-treated and control fruits. High carotenoids concentration in Put-treated fruit during NA storage was similar to previously reported in PAs-treated 'Kensington Pride' mango during 3 – 4 weeks of storage (Malik and Singh, 2005). However, the CA effect on retarding the synthesis of carotenoids contrasts with previously reported results by Lalel et al. (2001) in which carotenoids increased with increasing CO_2 (9%) during CA storage. This may be explained that lower CO_2 concentration (5%) in this research, which supressed the carotenoids synthesis during six weeks of storage.

At 2 weeks of storage, the decrease in ascorbic acid level was larger in Put-treated fruit than in fruit not treated with Put, regardless of the storage atmosphere composition. Those differences among treatments narrowed at 4 – 6 weeks of

storage. It has been reported that ascorbic acid content is higher in green mature than in ripe mango fruit (Gomez and Lalojo, 2008; Sulaiman and Ooi, 2012). Increase in ascorbic acid concentration has been reported during storage of Put-treated 'Kensington Pride' mango (Malik and Singh, 2005), and a decrease during ripening (Malik and Singh, 2006).

Total antioxidants level in mango pulp was preserved better by Put treatment combine with CA storage compared to Put treatment alone. It has been reported that green mature mango has higher antioxidant activity compared to ripe fruit and it varies among cultivars (Sulaiman and Ooi, 2012). The high total antioxidants content found in this research may indicate the effect of CA which delayed ripening process during storage at 13°C; however, the content of control fruit was also high.

In conclusion, Put treated fruit followed by CA storage generally retained fruit firmness and springiness, which negatively correlated to the *exo*-PG enzyme activity, retarded colour changes and pigments degradation, increased sugars and organic acids accumulation within 4 – 6 weeks of storage. However, the Put treatment alone did not exhibit better quality performance compared to control.

Chapter 7

Effects of 1-methylcyclopropene combined with controlled atmosphere storage on ripening quality of 'Kensington Pride' mango

Summary

Two experiments were conducted to evaluate the combined effect of 1-methylecyclopropene (1-MCP) treatment and controlled atmosphere storage on texture, pectolytic enzymes activities, colour development and other quality parameters in Kensington Pride' mango. In the first experiment, hard mature green mango (*Mangifera indica* L.) cv. 'Kensington Pride' fruit were treated with 1-MCP (0.6 $\mu l \cdot l^{-1}$ for 12 hours) and stored at 13 ± 0.5°C under normal air (NA) or controlled atmosphere (CA) storage containing 3% O_2 and 5% CO_2 for 3 and 5 weeks. In the second experiment, the fruit retrieved from CA storage after each storage period as the first experiment were ripened at 21 ± 1°C until a softness score of 4 ± 0.2 (eating soft, on a 1 – 5 score). The results showed that the combined treatment of 1-MCP and CA was beneficial in retarding fruit softening, *exo*- and *endo*-PG enzymes activities; delaying skin colour changes including chlorophylls degradation, and carotenoids synthesis; and postponing sugars accumulation with high retention in organic acids during storage. In addition, the synergistic effect of 1-MCP treatment and CA storage also improved the ripe quality of mango fruit by the development of yellowness associated to the reduction of chlorophylls, the increase in sugars accumulation while maintained its organic acids concentration, and the reduction of antioxidants loss following 3 weeks of storage. The 1-MCP treatment alone was effective to some extent, such as maintaining the sugar/acids ratio, and preserving carotenoids and total antioxidants level in ripe fruit. The combination effect of pre-storage treatment of 1-MCP and CA storage resembled to CA storage alone in overall quality of fruit during ripening following three weeks storage. Prolonged storage to five weeks resulted in low quality of ripe mango fruit. In conclusion, 'Kensington Pride' mango treated with 1-MCP could be stored in CA for 3 weeks with acceptable changes in fruit quality.

7.1.Introduction

Mango (*Mangifera indica* L.) is a climacteric fruit which exhibits climacteric peak of respiration together with high production of C_2H_4 during natural ripening (Singh and Singh, 2012). The process occurs within a short period of time after harvest, followed by physiological and biochemical changes such firmness, starch, acidity, colour, pigments and other compositional changes including nutritional value which shorten the storage and shelf life (Singh et al., 2013). For instance, firmness of 'Tommy Atkins' is completely altered within 9 days of postharvest (Medlicott et al., 1988). Mango fruit softening is associated with the degree of depolymerisation in cell wall polysaccharides, where a change in size and nature of those polymers is due to cell wall hydrolyses (Abu-Sarra and Abu-Goukh, 1992; Ali et al., 1990; Ali et al., 1995; Ali et al., 2004; Chourasia et al., 2006; Chourasia et al., 2008; Singh and Singh, 2012). Various efforts have been made in postharvest technology to overcome the fragility of this exotic fruit.

Storage is important to extend the period between production and consumption, including market supply and long distance transportation. However, over-ripening and senescence are problems during this period due to the C_2H_4 action which lessening the quantity and quality of fresh produces. 1-MCP is a compound known to possess the characteristic of controlling C_2H_4 metabolism in postharvest produces. The commercial application demonstrate the ability of 1-MCP to reduce the ripening process hence extend shelf-life while maintain the quality of fresh produces (Blankenship and Dole, 2003; Sisler and Serek, 1997; Watkins, 2008; Watkins and Miller, 2005; Yang and Hoffman, 1984). 1-MCP has been reported to inhibit C_2H_4 production and respiration, postpone mango fruit ripening hence delay the ripening-related softening and skin colour changes (Hofman et al., 2001; Jiang and Joyce, 2000; Lalel et al., 2003g; Singh et al., 2007; Singh and Singh, 2012). Delay in SSC accumulation, reduction in soluble pectin and the degree of solubilised pectin is reported in connection to the inhibition of fruit softening enzyme by 1-MCP treatment in several mango cultivars (Hojo et al., 2006; Rangel et al., 2009; Razzaq et al., 2015; Singh and Neelam, 2008). During storage, ascorbic acid and β-carotene are also preserved by 1-MCP treatment (Bomfim et al., 2011; Islas-Osuna et al., 2010). However, several factors have been reported to contribute to the effectiveness of 1-MCP treatment i. e., exposure time, temperature, concentration, storage

atmosphere composition and cultivars affects the response of mango fruit to 1-MCP treatment (Singh and Singh, 2012; Sisler and Serek, 1997, 2003; Watkins, 2008). Low temperature treatment followed by 1-MCP application reduced the rate of de-greening but when the treatments were in reversed order, titratable acid was accumulated (Lima et al., 2007). The combination of technologies must be considered to create a conducive environment to extend storage- and shelf-life while eliminating unfavourable effects of the single one on overall quality of fresh produces.

Controlled atmosphere (CA) storage has been firstly reported by Singh et al. (1937) to prolong the storage life of mango fruit. CA reduces most of detrimental changes caused by ripening process (Beaudry, 1999; Mathooko, 1996a, b; Reid, 1992), including softening and de-greening in various mango cultivars (Brecht et al., 2003; Lalel et al., 2003a; Kim et al., 2007). 1-MCP pre-storage treatment in combination with CA condition reduces browning in apple (Nock and Watkins, 2013) and litchi (Sivakumar and Korsten, 2010). Singh and Singh (2012) reported that the chilling injury in plum tissue was reduced as the result of synergistic effect of 1-MCP treatment and CA storage. However, the degradation of pectin, which caused softening, can not be prevented in 1-MCP-treated peach fruit stored in CA (Ortiz et al., 2011). Hot water treated 'Kent' mango exposed to 1-MCP and stored in CA at different composition (5% O_2 + 5% CO_2 and 3% O_2 + 8% CO_2) resulted in diverse quality level and duration of postharvest life. Higher CO_2 composition in combination with 1-MCP treatment showed better prevention of anthracnose severity level, retention of fruit firmness, reduction of colour development of both skin and flesh, postponement of the increase of SSC/TA ratio, preservation of ascorbic acid and antioxidant scavenging activity compared to lower one. Although the quality of ripe fruit were not significantly different at 10 days of ripening, the combination of 1-MCP treatment and CA (3% O_2 + 8% CO_2) resulted in lower ethanol and acetaldehyde production hence reduced the off-flavour compared to other treatments (Sivakumar et al., 2012). Under anaerobic conditions, the senescence and ethylene production are inhibited due to high concentration of ethanol and acetaldehyde (Pesis, 2005) which may reduce the eating quality of fruit. It was assumed that lower CO_2 (5%) concentration during storage may improve the quality of 'Kensington Pride' mango fruit treated with 1-MCP. A comprehensive analysis of quality of

'Kensington Pride' mango fruit treated with 1-MCP during storage under CA condition followed by ripening at ambient temperature is lacking, and it warranted investigation.

7.2. Materials and methods

7.2.1. Fruit material

Hard mature green 'Kensington Pride' mangoes were obtained from a commercial orchard located at Gingin (long. 115°55'E, lat. 31°21'S), Western Australia in March 2008. Fruit were dipped for two minutes in an aqueous fungicide solution containing 0.55 $ml \cdot l^{-1}$ 'Sportak' (prochloraz as an active ingredient), air dried, packed in the soft-board, and transported by air conditioned vehicle (15 ± 1°C) to the Horticultural Research Laboratory, Curtin University, Perth, Western Australia. Uniformly mature fruit, free from visible symptoms of any diseases or blemishes were used in the experiments.

7.2.2. Experiments

7.2.2.1. *Experiment 1:* Effect of pre-storage treatment of 1-MCP on fruit softening, colour development and nutritional quality in 'Kensington Pride' mango during controlled atmosphere storage

SmartFresh™ SmartTabs tablets (AgroFresh, Inc., Rohm and Hass Australia Pty., Ltd., Victoria, Asutralia) were mixed with the blue activator tablets and solution to vaporized its active ingredient, 1-MCP. Fruit were treated with 0.6 $\mu l \cdot l^{-1}$ 1-MCP for 12 h at 13°C under an air-tight plastic tend equipped with mini-electric fans for distributing 1-MCP gas evenly. The 1-MCP treated, and untreated fruit were stored in normal atmosphere (NA) and in 90-litre chambers of controlled atmosphere (CA) composed of 3% O_2 and 5% CO_2 at 13 ± 0.5°C and 85 ± 3% RH. Concentration of O_2 and CO_2 in the CA chambers were adjusted with N_2. The CA storage was a continuous gas flow with open ended system, and the conditions were maintained and monitored by a Gas Analyser (ADC 7000 series, Analytical Development Company Ltd., Hoddesdon, Herts, UK). A single chamber containing 10 fruit was treated as one treatment unit and replicated three times. The fruit were removed from storage after 3 and 5 weeks of storage.

7.2.2.2. *Experiment 2:* The quality of ripe 'Kensington Pride' mango as affected by 1-MCP treatment combined with CA storage

After receiving in laboratory, fruit were subjected to 1-MCP treatment as described in 7.2.2.1 and stored in CA (3% O_2 + 5% CO_2) at 13 ± 0.5°C and 85 ± 3% RH. Ripening process was conducted after 3 and 5 weeks of CA storage at ambient condition (temperature: 21 ± 1°C, RH: 58.02 ± 7.02 %). Two sets of fruit, with and without 1-MCP treatment, were placed on shelf (0 weeks of storage) at room temperature of 21 ± 1°C (RH: 58.02 ± 7.02%) until 'eating soft' (i.e. based on a softness score of 4 on a 1 – 5 scale, as described in section 3.3.1) as 1-MCP-treated and control fruit, respectively. The experiment was laid out by following a completely randomised design with 5 fruit as an experimental unit and replicated 3 times.

7.2.3. Analysis of fruit texture

The non-destructive measurement on fruit softness were followed the method described in section 3.3.1. Destructive method to measure fruit firmness was done by texture analyser as previously described in section 3.3.2.2 where fruit were cut at the equatorial area by 2 x 2 cm^2 in width x height.

7.2.4. Determination of fruit softening enzymes activities

The activities of softening enzymes i.e., *exo*-polygalacturonase (*exo*-PG), *endo*-polygalacturonase (*endo*-PG), and pectin esterase (PE) were determined in pulp tissues accordingly as described in section 3.4. Cryogenically frozen fresh fruit pulp stored at -80°C was used for enzyme analysis.

7.2.5. Protein determination

Protein content in pulp tissues was determined following the method of Bradford (1976) with some modification. Detailed procedure described in section 3.4.3.

7.2.6. Fruit colour assessment

Fruit were visually assessed for skin colour following the method as explained in section 3.5.1. The data was recorded after 3 and 5 weeks in the experiment 1, and after 3 and 5 weeks of storage followed by daily assessment to

fully ripe in the experiment 2. The objective colour assessment was exercised as detailed in section 3.5.2.

7.2.7. Determination of skin pigments concentration

Method developed by Lichtenthaler (1987) was followed in the determination of fruit skin colour with some modifications as detailed in section 3.6.

7.2.8. Determination of sugars and organic acids

High performance liquid chromatography (HPLC Waters, Milford, MA, USA) was used in sugars and organic acids determinations. Individual sugars (sucrose, fructose and glucose) and organic acids (citric, malic, and succinic acids) were extracted from mango pulp tissues, and injected to HPLC for separation and determination. External standards of sugars and organic acids were run through HPLC system for calibration and calculation. Sugars and organic acids content were expressed in $\mu g \cdot g^{-1}$ FW pulp. Detailed procedure described in sections 3.7.1 and 3.7.2.

7.2.9. Determination of total carotenoids, ascorbic acid, and total antioxidants

Mango pulp total carotenoids, ascorbic acid, and total antioxidants were estimated as detailed in section 3.8 to 3.10.

7.2.10. Statistical analysis

The effects of various treatments and storage period, or treatments and ripening period were assessed as detailed in section 3.11.

7.3. Results

7.3.1. *Experiment 1:* Effect of pre-storage treatment of 1-MCP on fruit softening, colour development and nutritional quality in 'Kensington Pride' mango during controlled atmosphere storage

7.3.1.1. Fruit texture profiles and softness during storage

Storage period significantly ($P \leq 0.05$) affected fruit firmness, springiness, and gumminess but not other texture parameters (Table 7.1). Fruit firmness decreased significantly during 6 weeks in CA storage, but 1-MCP treated fruit stored under NA condition did not show significant decrease in firmness after 3 to 4 weeks

of storage. However, 1-MCP-treated fruit stored in CA or in NA did not perform better firmness than CA-stored fruit (Table 7.1).

Decreasing fruit firmness was in accordance to increasing fruit softness during storage (Figure 7.1.). Regardless of 1-MCP treatment, fruit firmness declined from its initial value about 96% and 97% while softness increase about 70% and 79% in fruit stored in CA and NA, respectively after 5 weeks of storage. The duration of storage significantly affected fruit softness. Fruit stored under NA showed significantly ($P \leq 0.05$) higher softness score compared to those stored in CA, regardless of 1-MCP treatment.

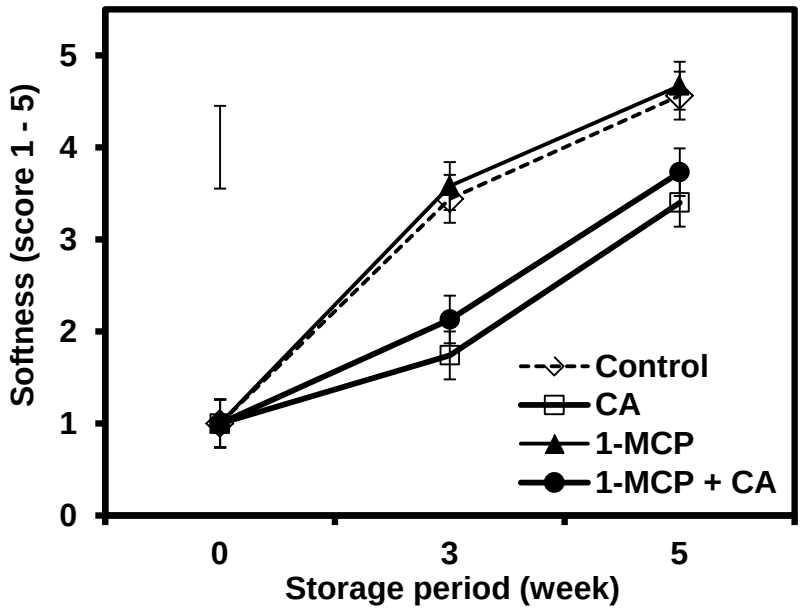

Figure 7.1. Changes in subjective softness of 'Kensington Pride' mangoes during storage (SP) as influenced by 1-MCP and CA storage (T) at 13 ± 0.5°C for 3 and 5 weeks. Vertical bars within graphs represent SEM (n = 15; 5 fruit x 3 replications), vertical bar outside graph represent LSD ($P \leq 0.05$). LSD values for T = 0.26, SP = 0.22, T x SP = 0.45.

Table 7.1. Texture profile analysis (TPA) of 'Kensington Pride' mangoes before (0 week) and after 3 and 5 weeks of storage at 13°C in normal atmosphere as control and controlled atmosphere (CA: 3% O_2 + 5% CO_2), treated with and without 1-MCP

Texture profile	Storage period (weeks)	Control			CA			1-MCP			1-MCP + CA			*Means* (SP)	LSD ($P \leq 0.05$)
Firmness (N)	0	119.37	a	A	119.37	a	A	119.37	a	A	119.37	a	A	*119.37*	T = ns
	3	5.57	bc	B	7.05	a	B	5.37	c	B	6.05	b	B	*6.01*	SP = 0.39
	5	4.50	b	C	4.01	bc	C	4.81	a	B	4.12	c	C	*4.36*	T x SP = ns
Means (T)		*43.15*			*43.48*			*43.18*			*43.18*			*43.25*	
Cohesiveness	0	0.06	a	A	0.06	a	A	0.06	a	AB	0.06	a	A	*0.06*	T = ns
	3	0.05	a	A	0.03	b	A	0.04	a	B	0.04	ab	B	*0.04*	SP = ns
	5	0.04	b	A	0.03	b	A	0.08	a	A	0.02	b	C	*0.04*	T x SP = ns
Means (T)		*0.05*			*0.04*			*0.06*			*0.04*			*0.05*	
Springiness (mm)	0	2.96	a	A	2.96	a	A	2.96	a	A	2.96	a	A	*2.96*	T = ns
	3	1.62	a	B	1.14	b	B	1.42	ab	B	1.49	a	B	*1.42*	SP = 0.29
	5	1.08	b	B	0.93	b	B	1.97	a	B	0.88	b	C	*1.22*	T x SP = ns
Means (T)		*1.89*			*1.68*			*2.12*			*1.78*			*1.87*	
Gumminess (N)	0	7.40	a	A	7.40	a	A	7.40	a	A	7.40	a	A	*7.40*	T = ns
	3	0.30	a	B	0.19	b	B	0.24	ab	B	0.27	ab	B	*0.25*	SP = 0.86
	5	0.18	b	B	0.11	b	B	0.38	a	B	0.10	b	B	*0.19*	T x SP = ns
Means (T)		*2.63*			*2.57*			*2.67*			*2.59*			*2.61*	
Fracture force (N)	0	1.53	a	A	1.53	a	A	1.53	a	A	1.53	a	A	*1.53*	T = ns
	3	1.19	a	A	1.07	a	B	1.44	a	A	1.05	a	B	*1.19*	SP = ns
	5	1.85	a	A	1.21	a	AB	1.43	a	A	1.03	a	B	*1.38*	T x SP = ns
Means (T)		*1.52*			*1.27*			*1.47*			*1.20*			*1.37*	

Texture profile	Storage period (weeks)	Control			CA			1-MCP			1-MCP + CA			*Means* (SP)	LSD ($P \leq 0.05$)
Adhesive force (N)	0	0.60	a	A	0.60	a	A	0.60	a	A	0.60	a	AB	*0.60*	T = ns
	21	0.37	a	A	0.43	a	A	0.21	b	B	0.30	ab	B	*0.33*	SP = ns
	35	0.31	b	A	0.36	b	A	0.29	b	AB	0.69	a	A	*0.41*	T x SP = ns
Means (T)		*0.42*			*0.46*			*0.37*			*0.53*			*0.45*	

n = 15 (5 fruit x 3 replications), ns = not significant at $P \leq 0.05$, 1-MCP = 1-methylcyclopropene treatment stored in NA, 1-MCP + CA = 1-methylcyclopropene treatment combined with controlled atmosphere storage, T = treatment, SP = storage period. Values within a row followed by the same lowercase letter(s) are not significantly different and values within a column followed by the same uppercase letter(s) are not significantly different at $P \leq 0.05$.

7.3.1.2.Fruit softening enzymes activities

The duration of storage and 1-MCP treatment significantly ($P \leq 0.05$) affected *endo*-PG activity in mango pulp. After 3 weeks of storage, the activity of *endo*-PG decrased significantly in all fruit, irrespective of treatments. However after 5 weeks in storage, the *endo*-PG activity increased about 10-fold in control fruit held under NA whereas in CA-stored fruit increased about 5 fold and no change in *endo*-PG activity was observed in 1-MCP-treated fruit stored in NA (Figure 7.2.A). The *endo*-PG activity was significantly higher in untreated fruit held in NA compared to untreated CA-stored fruit, 1-MCP-treated kepted in NA, and 1-MCP-treated fruit combined with CA storage; and it was 3.7, 20.0, and 4.4-fold higher, respectively. The interaction between storage period and 1-MCP treatment significantly ($P \leq 0.05$) influenced the *endo*-PG activity. The activity of *exo*-PG in mango pulp was significantly ($P \leq 0.05$) influenced by storage treatments and storage duration (Figure 7.2.B). During the first 3 weeks of storage, the activity was declined between 13 – 21% from the initial activity but the differences among treatments were not significant. *Exo*-PG activity increased significantly after five weeks of storage in untreated mango pulp compared to other treatments. The activity of *exo*-PG in control fruit was measured at 1.3, and 1.2 fold higher than the activity in CA-stored fruit, and 1-MCP-treated fruit stored in NA and CA, respectively.

The initial activity of PE was high in mango pulp (0.84 mM NaOH·mg protein^{-1}· h^{-1}), and it decreased during three weeks of storage about 91% in untreated fruit stored in NA while in others about 93%. There were no differences among treatments at either 3 or 5 weeks of storage (Figure 7.2.C).

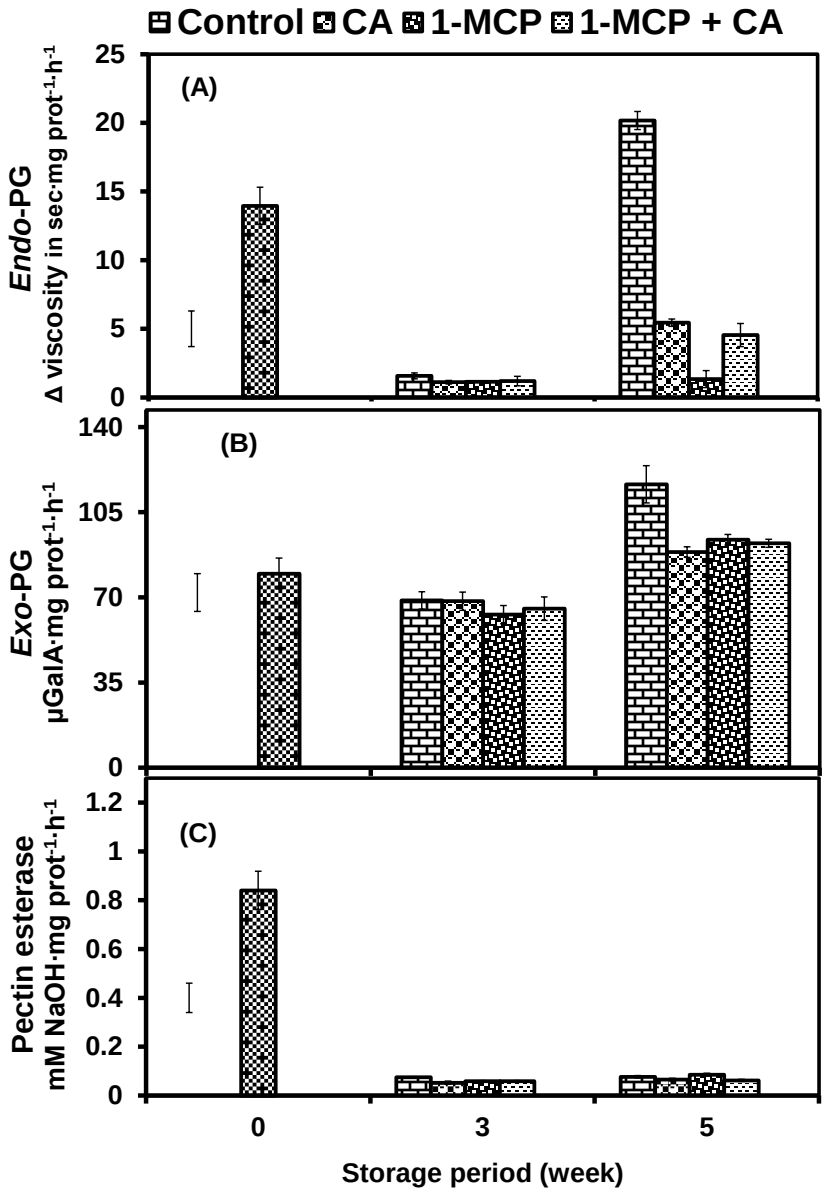

Figure 7.2. Changes in activities of *endo*- (A), *exo*-PG (B), and PE (C) enzymes in the flesh tissue of 'Kensington Pride' mangoes as influenced by 1-MCP and CA storage (T) at 13 ± 0.5°C for 3 and 5 weeks (SP). Vertical bars within graphs represent SEM, n = 3 replications, vertical bar outside graph represent LSD ($P \leq 0.05$). LSD for *endo*-PG: T = 1.46, SP = 1.27, T x SP = 2.53; *exo*-PG: T = ns, SP = 7.75, T x SP = ns; PE: T = ns, SP = 0.07, T x SP = ns

7.3.1.3.Fruit skin colour and pigments

Overall colour of 1-MCP-treated 'Kensington Pride' mango was significantly ($P \leq 0.05$) influenced by storage treatments, and duration of storage. Retention of green colour in fruit kept under CA was significantly higher during 3 and 5 weeks of storage at 13 ± 1°C, showed by low a*, b*, and visual colour score, and high hue angle (Figure 7.3). The lightness (L*) of CA-stored fruit was also maintained lower than fruit kept in NA for 3 and 5 weeks. No significant changes in colour attributes were observed in CA-treated fruit, regardless of 1-MCP treatment. Substantial changes in colour were evaluated in fruit stored in NA.

After 3 weeks of storage, the a* value of NA-stored fruit increased 2-fold from its initial value which was in accordance with the huge leap of visual colour score from 1 to 4.7 and 5 in 1-MCP-treated and untreated fruit, respectively. CA-stored fruit retained the initial colour but NA-treated fruit experienced degradation of its green during five weeks of storage, irrespective of 1-MCP treatment (Figure 7.3).

The changes of pigments concentration in skin of 1-MCP-treated 'Kensington Pride' mango as influenced by storage treatments and storage duration are presented in Figure 7.4. The storage atmosphere, storage period and their interaction significantly affected chlorophylls and catorenoids content in mango skin. Chlorophyll a and b were observed to be significantly increased in fruit stored in CA with or without 1-MCP treatment after 3 weeks of storage, which were 1.5 and 2 fold, respectively. In contrast to CA-stored fruit, about half of the initial chlorophylls content in fruit stored in NA was reduced at the same storage duration; and further reduction was noticed after five weeks. A significant higher retention of chlorophylls was recorded in mango skin kept in CA compared to 1-MCP-treated fruit in the same atmosphere condition and all fruit kept in NA for three weeks. After five weeks of storage, the chlorophylls accumulated in CA-stored fruit were about 2-fold whereas that left in NA-stored fruit with and without 1-MCP treatment were 11 and 17 %, respectively from the initial concentration.

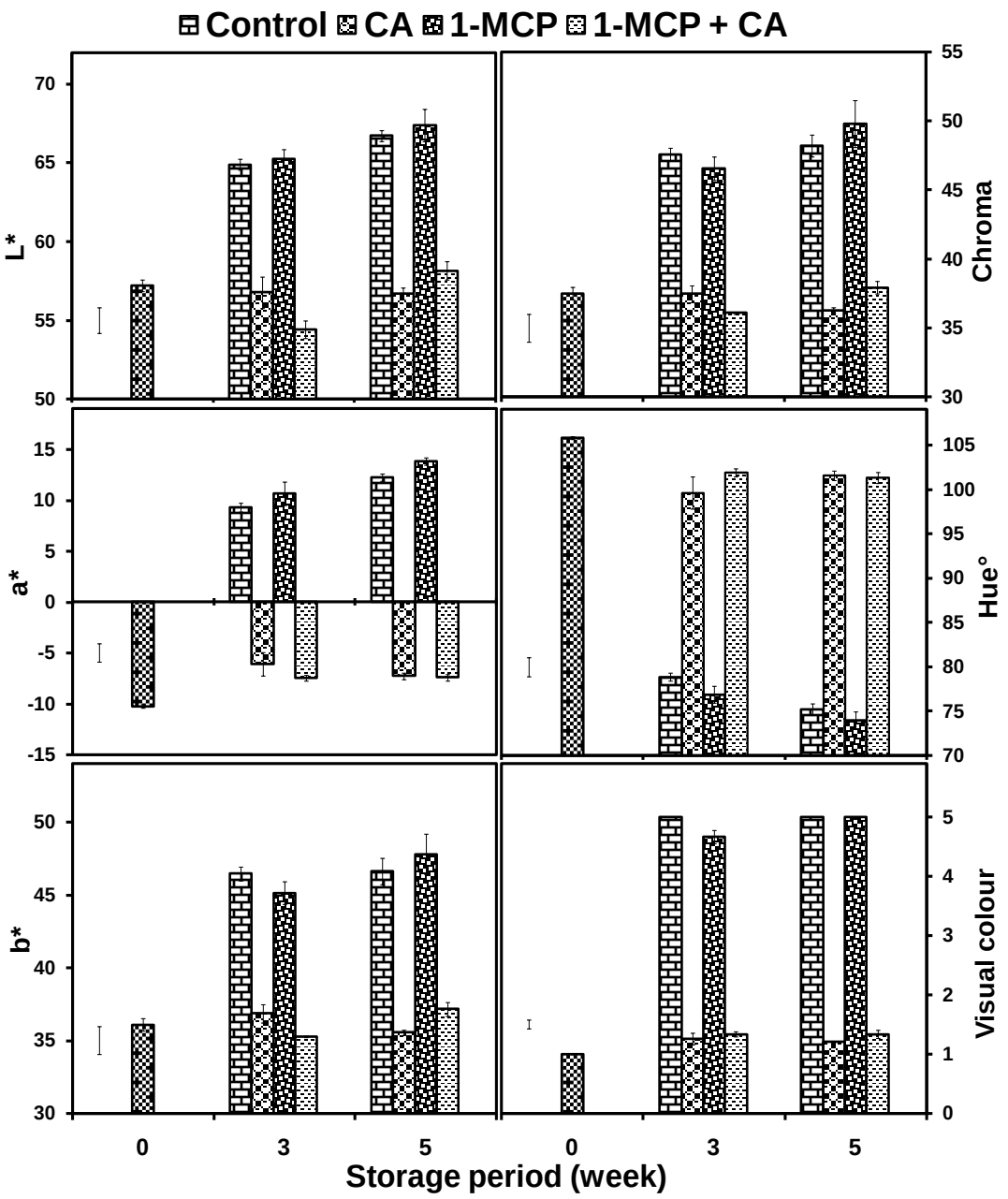

Figure 7.3. Changes in skin colour (L*, a*, b*, chroma, hue angle, and visual colour) of 'Kensington Pride' mangoes as influenced by 1-MCP and CA storage (T) at 13 ± 0.5°C for 3 and 5 weeks (SP). Vertical bars within graphs represent SEM (n = 15; 5 fruit x 3 replications), vertical bar outside graph represent LSD ($P \leq 0.05$). LSD for L*: T = 0.98, SP = 0.83, T x SP = 1.66; a*: T = 1.02, SP = 0.89, T x SP = 1.77; b*: T = 1.09, SP = 0.94, T x SP = 1.89; chroma: T = 1.18, SP = 1.02, T x SP = 2.04; Hue°: T = 1.25, SP = 1.09, T x SP = 2.18; visual colour: T = 0.08, SP = 0.07, T x SP = 0.14.

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Carotenoids significantly ($P \leq 0.05$) accumulated in all fruit after three weeks of storage but there were no significant differences among treatments (Figure 7.4). Further changes in carotenoids level were measured after 5 weeks of storage. While the control fruit constantly accumulated carotenoids and 1-MCP-treated fruit maintained its content after 5 weeks of storage, the CA-stored fruit were unable to keep the carotenoids content higher than NA-stored fruit.

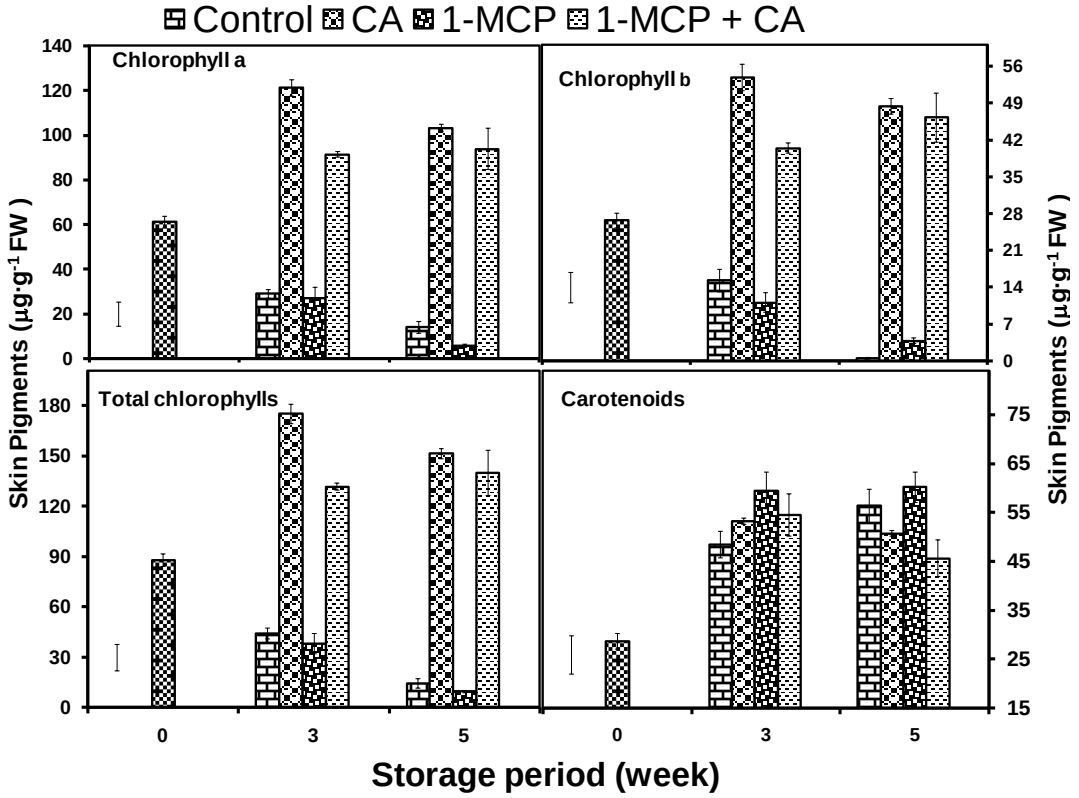

Figure 7.4. Changes in fruit skin pigments (chlorophyll a, b, total chlorophylls and carotenoids) of 'Kensington Pride' mangoes as influenced by 1-MCP and CA storage (T) at 13 ± 0.5°C for 3 and 5 weeks (SP). Vertical bars within graphs represent SEM, n = 3 replications, vertical bar outside graph represent LSD ($P \leq 0.05$). LSD for chlorophyll a: T = 6.15, SP = 5.33, T x SP = 10.65; chlorophyll b: T = 3.35, SP = 2.90, T x SP = 5.80; total chlorophylls: T = 9.02, SP = 7.81, T x SP = 15.62; carotenoids: T = 4.52, SP = 3.91, T x SP = 7.83.

7.3.1.4.Sugars and organic acids composition

The sucrose and glucose concentration of 'Kensington Pride" mangoes were significantly influenced by treatment and duration of storage; whereas the concentrations of fructose and total sugars were significantly affected by storage duration (Figure 7.5.A, and B for data after 3 and 5 weeks of storage, respectively). The interaction effect of treatment and storage period on sugars accumulation in mango pulp was significant.

The accumulation of sucrose significantly increased after three weeks of storage in all fruit which was about 2 and 3-fold in CA- and NA-stored fruit, respectively, regardless of 1-MCP treatment (Figure 7.5.A). However, as the storage proceeded to five weeks, the sucrose concentration was decreased in all fruit (Figure 7.5.B). Higher reduction in sucrose was measured in 1-MCP-treated fruit stored in NA compared to CA-stored fruit, with or without 1-MCP treatment. The combination between 1-MCP and CA storage resulted in significantly lower sucrose after five weeks of storage compared to others, although its rate of reduction was lower than fruit stored in NA. Fructose concentration significantly increased after three weeks of storage in all fruit, and there were no significant differences among treatments. With an exception of control fruit, further significant increase was measured after five weeks of storage with higher concentration in CA-treated fruit, regardless of 1-MCP treatment. Glucose concentration was found the lowest to contribute to total sugars. The accumulation of glucose in 1-MCP-treated fruit stored in CA was noticed after five weeks of storage whereas tracer amount was detected in others. In total, the sugars content significantly increased after three weeks in all fruit. Extension of storage to five weeks resulted in significant decrease in total sugars, which mostly due to the declining concentration of sucrose.

Treatments, storage durations and their interactions significantly influenced organic acids composition in fruit pulp. The concentration of citric and malic acid changed significantly after three weeks of storage, and there were significant difference among treatments ($P \leq 0.05$) (Figure 7.6.A). Succinic acid contributes the largest amount of total organic acids concentration in mango pulp. Succinic acid concentration was higher in CA-stored fruit without 1-MCP treatment followed by 1-MCP-treated fruit stored under CA, than all fruit stored under NA, which was about 2.7, 1.9, and 1.4-fold, respectively. Further significant increase in succinic acid was

observed in 1-MCP-treated fruit stored for five weeks in CA storage, which similar to control fruit (Figure 7.6.B). In contrast to succinic acid, the concentration of citric acid declined after 3 weeks of storage, which was about 84 and 83% in control and in 1-MCP-treated fruit kept in NA, and 46 and 20% in CA-stored fruit treated with and without 1-MCP, respectively. After five weeks in CA storage, the citric acid decreased about 45% while fruit stored at the same atmosphere but treated with 1-MCP produced more citric acid. There were significant differences among treatments where 1-MCP together with CA storage caused higher accumulation of citric acid, followed by CA storage, than 1-MCP treatment in NA and control fruit. A significant decrease in malic acid content during CA storage occurred after five weeks of storage while that combined with 1-MCP treatment took place two weeks earlier. 1-MCP treatment alone significantly accumulated malic acid after 3 weeks of storage and maintained its level until 5 weeks which resulted in higher malic acid than control and CA storage. In general, changes in total organic acids were significantly influenced by the treatments, storage duration, and the interaction between them.

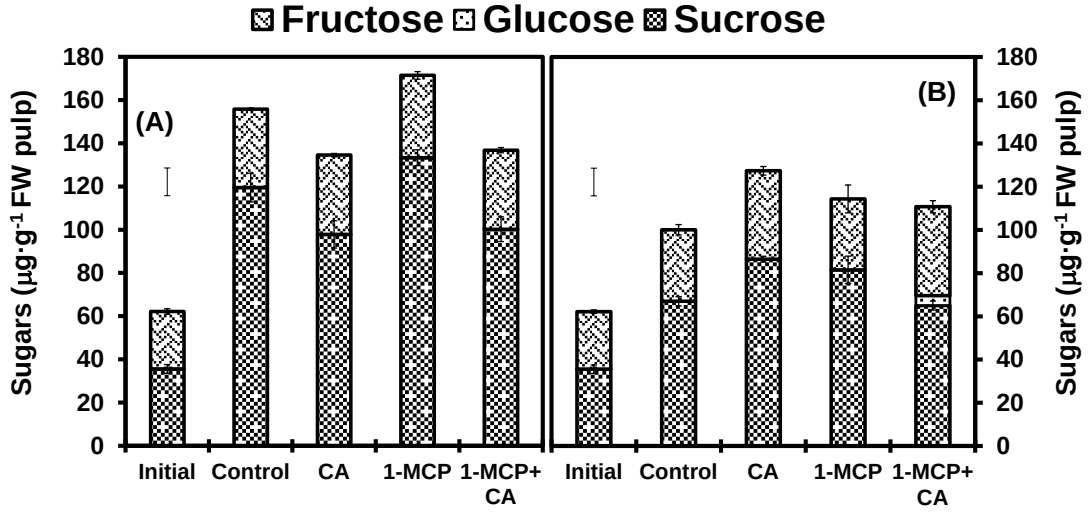

Figure 7.5. Changes in sugars concentration of 'Kensington Pride' mangoes as influenced by 1-MCP and CA storage (T) at 13 ± 0.5°C for 3 (A) and 5 (B) weeks (SP). Vertical bars outside graphs represent LSD ($P \leq 0.05$). Vertical bars within graphs represent SEM, n = 3 replications, LSD ($P \leq 0.05$) for sucrose: T = 6.83, SP = 5.92, T x SP = 11.83; glucose: T = 0.05, SP = 0.04, T x SP = 0.08; fructose: T = ns, SP = 1.57, T x SP = 0.04; Total sugars: T = ns, SP = 6.41, T x SP = 12.82.

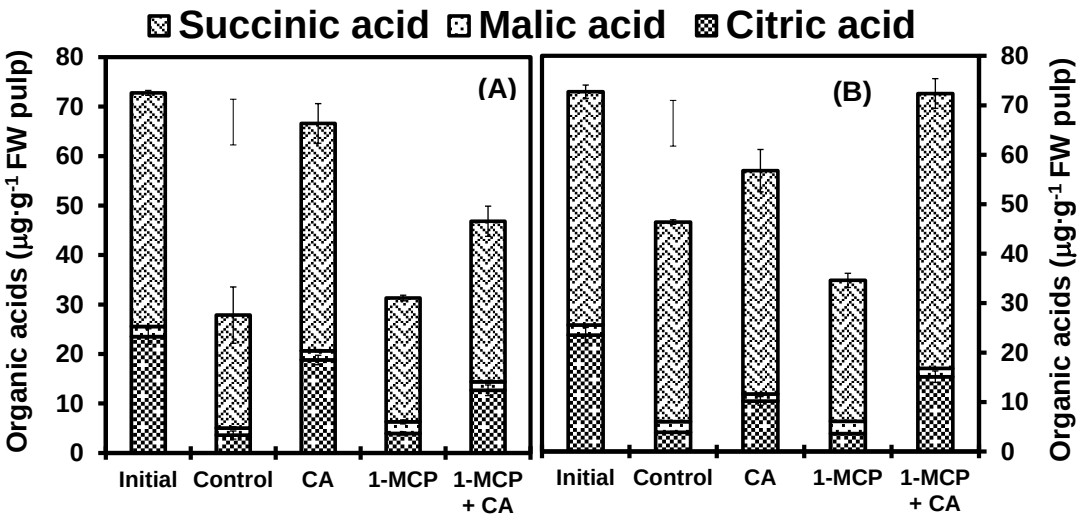

Figure 7.6. Changes in organic acids concentration of 'Kensington Pride' mangoes as influenced by 1-MCP and CA storage (T) at 13 ± 0.5°C for 3 (A) and 5 (B) weeks (SP). Vertical bars outside graphs represent LSD ($P \leq 0.05$). Vertical bars within graphs represent SEM, n = 3 replications, LSD ($P \leq 0.05$) for succinic acid: T = 4.26, SP = 3.69, T x SP = 7.37; malic acid: T = 0.33, SP = 0.29, T x SP = 0.57; citric acid: T = 1.87, SP = 1.62, T x SP = 3.24; Total organic acids: T = 5.34, SP = 4.62, T x SP = 9.24.

7.3.1.5.Carotenoids, ascorbic acid, and total antioxidants

Carotenoids, ascorbic acid, and total antioxidants in 'Kensington Pride' mangoes were significantly influenced by treatments and duration of storage (Figure 7.7). The interaction effect of treatments and storage period was significant at $P \leq 0.05$. The pulp carotenoids in all fruit increased significantly after three weeks of storage with the highest concentration was evaluated in control fruit followed by 1-MCP treatment combined with CA storage, 1-MCP treatment, and CA storage. Prolonged storage duration caused a further increase in carotenoids in CA-stored fruit, and a significant decrease in 1-MCP-treated fruit stored under CA and in control fruit, while 1-MCP treatment alone maintained its carotenoids level similar to the third week of storage. The carotenoids content in 1-MCP-treated fruit combined with CA storage decreased to the greater extent compared to control, resulted in lower concentration compared to other treatments after five weeks of storage.

The ascorbic acid concentration in mango fruit significantly decreased after three weeks of storage, but the reduction was significantly higher in all NA-stored fruit than in CA-stored fruit, regardless of 1-MCP treatment (Figure 7.7). As the

storage proceeds to five weeks, the CA-stored fruit kept the ascorbic acid at higher level compared to NA-stored fruit, whereas significant decrease was observed in CA-stored fruit without 1-MCP treatment. The determination of ascorbic acid after five weeks of storage showed that fruit held in CA storage with 1-MCP treatment contained significantly higher ascorbic acid that CA storage alone.

As influenced by storage duration, the total antioxidants in mango pulp decreased significantly after three weeks of storage but lower level of decrease was observed in fruit held in CA than in NA. However, lower total antioxidant activity was noted in 1-MCP-treated fruit kept in NA compared to fruit stored in CA without 1-MCP treatment during the third week of storage; and it was also lower than control. The extension in storage duration to five weeks resulted in minor change of total antioxidant activity. The antioxidant activity in mango is primarily contributed by ascorbic acid; therefore the pattern of changes resembled that in ascorbic acid during 3 and 5 weeks of storage (Figure 7.7).

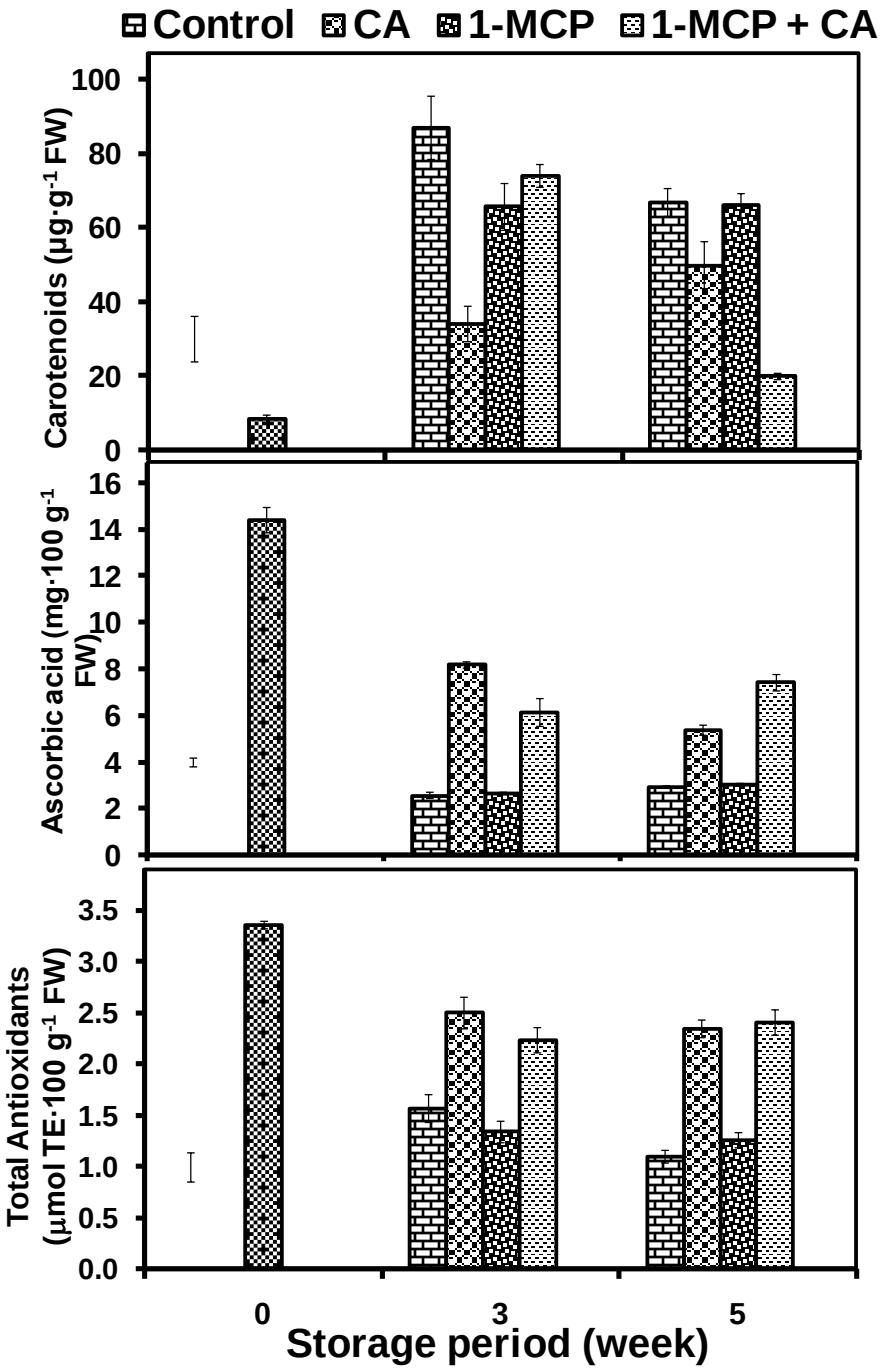

Figure 7.7. Changes in carotenoids, ascorbic acid, and total antioxidants concentration of 'Kensington Pride' mangoes as influenced by 1-MCP and CA storage (T) at 13 ± 0.5°C for 3 and 5 weeks (SP). Vertical bar outside graphs represent LSD ($P \leq 0.05$). Vertical bars within graphs represent SEM (n = 3 replications), LSD ($P \leq 0.05$) for carotenoids: T = 7.10, SP = 6.15, T x SP = 12.30; ascorbic acid: T = 0.23, SP = 0.20, T x SP = 0.39; Total antioxidants: T =

7.3.2. *Experiment 2:* The quality of ripe 'Kensington Pride' mango as affected by 1-MCP treatment combined with CA storage

7.3.2.1. Fruit texture

During ripening, the softness of fruit changed significantly. The ripening process was terminated when the softness was scored at 4 ± 0.2 which was considered as 'eating soft'. The 'eating soft' state of mango fruit kept at ambient condition was accomplished after 9 days whereas for fruit stored under CA for 3 and 5 weeks, that state was achieved after 6 and 2 days, respectively. 1-MCP treatment did not affect the softness of fruit kept in ambient condition although the score was lower than control ones. Ripening period significantly affected fruit softness under this condition but no significant interaction between treatment and ripening period. The influence of 1-MCP treatment was significantly noticed during ripening of 3 weeks CA-stored fruit where 1-MCP-treated fruit showed higher softness during five days of ripening and remained stable afterwards while the untreated fruit continued to soften (Figure 7.8). No interaction effect was determined between treatment and ripening period in all fruit.

Fruit firmness decreased as the softness increased (Figure 7.9). The firmness of all fruit stored under CA for three weeks was significantly higher than those stored for five weeks. During ripening, 1-MCP treatment lessened the reduction of firmness better than CA storage alone, resulted in no significant difference of firmness of the ripe fruit. The firmness of ripe fruit decreased significantly ($P \leq 0.05$), and it was about 18 and 29 % lower than the initial firmness measured immediately after three weeks of storage in 1-MCP-treated and untreated fruit, respectively. Comparing the ripe fruit firmness, the firmness of CA-stored fruit was lower than fruit in shelf although the firmness of CA-stored fruit measured immediately after three weeks of storage was higher than ripe fruit in shelf. The firmness of ripe fruit after 3 and 5 weeks of CA storage was not significantly different. After five weeks of CA storage, the firmness of the ripe fruit increased non-significantly, irrespective of the 1-MCP treatment.

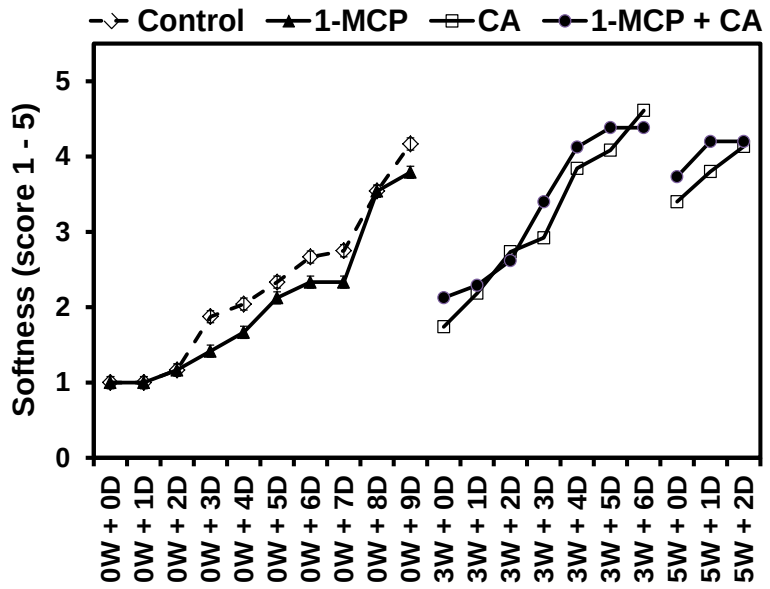

Figure 7.8. Changes in fruit softness of 'Kensington Pride' mangoes during ripening (RP) at 21 ± 1°C without storage (0W), and after 3 and 5 weeks of storage (3W and 5W, respectively), as influenced by 1-MCP and CA (T). Vertical bars within graphs represent SEM (n = 15; 5 fruit x 3 replications). LSD ($P \leq 0.05$) for softness at 0W: T = ns, RP = 0.32, T x RP = ns; 3W: T = 0.10, RP = 0.18, T x RP = ns; 5W: T = ns, RP = ns, T x RP = ns. RP = ripening period, W = weeks of storage; D = days of ripening period.

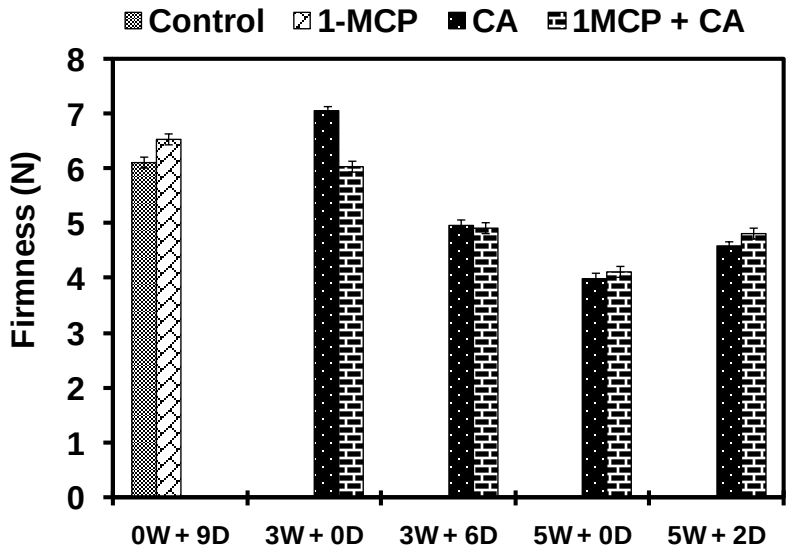

Figure 7.9. Changes in firmness (N) of 'Kensington Pride' mangoes during ripening (RP) at 21 ± 1°C without storage (0W), and after 3 and 5 weeks of storage (3W and 5W, respectively), as influenced by 1-MCP and CA (T). Vertical bars within graphs represent SEM (n = 15; 5 fruit x 3 replications). LSD ($P \leq 0.05$) for Firmness: T = 0.10, RP = 0.18, T x RP = ns. RP = ripening period, W = weeks of storage, D = days of ripening period.

7.3.2.2. Fruit softening enzymes activity

The activities of *endo*-, *exo*-PG and PE were significantly influenced by ripening period but only *endo*-PG activity was significantly affected by treatment and the interaction between treatment and ripening period (Figure 7.10). Within 9 days in shelf at room temperature, 1-MCP treatment depressed the activity of *endo*-PG in ripe fruit whereas in control the activity was 4 times higher. However, the *endo*-PG activity inclined significantly during ripening of 3-week CA-stored fruit as much as 10-fold in fruit without 1-MCP, and 7.8-fold in fruit with 1-MCP treatment. The increasing activity of *endo*-PG resulted in 1.2-fold higher in untreated-CA-stored fruit than in 1-MCP-treated combined with CA storage. Contrarily, the untreated fruit stored for five weeks in CA showed a significant reduction in *endo*-PG activity during ripening while the 1-MCP-treated fruit kept its activity at the same level as the day the fruit were retrieved from the storage.

A significant increase in e*xo*-PG activity was noticed during ripening of all CA-stored fruit. The increase in storage duration caused a further increase in the *exo*-PG activity during ripening of 1-MCP-treated fruit which was 1.6 and 1.3-fold after 3 and 5 weeks of storage intervals, respectively; and it was higher than CA-stored fruit without 1-MCP treatment. The ripe 1-MCP-treated fruit showed 1.3 fold higher *exo*-PG activities than 1-MCP-untreated fruit after three weeks of storage. The PE activity in ripe fruit was not significantly affected by 1-MCP treatment, but the activity increased significantly as the extension in storage, resulted in higher PE activity in ripe fruit stored for five weeks under CA condition.

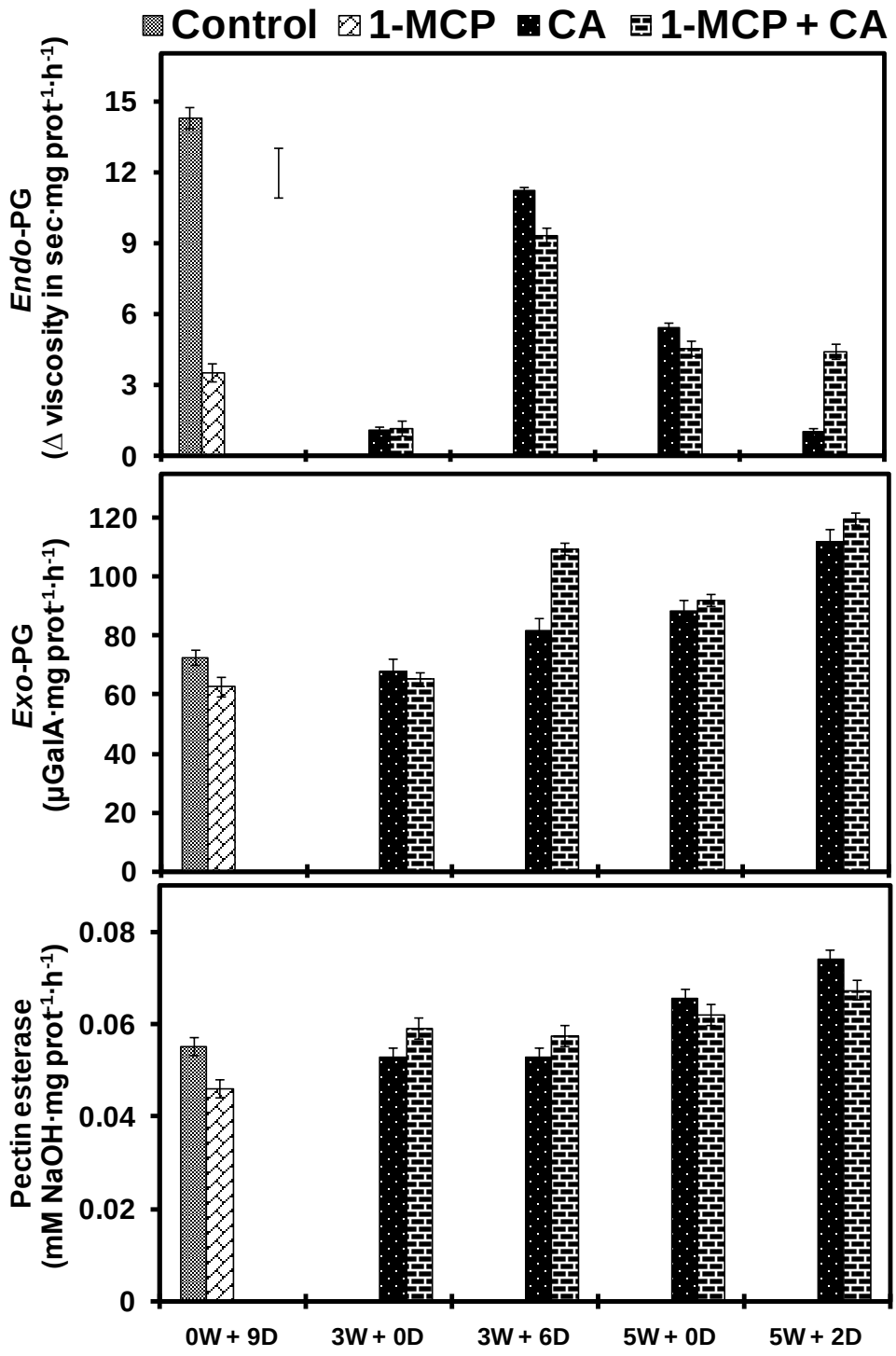

Figure 7.10. Changes in *endo*-, *exo*-PG, and PE activities of ripe 'Kensington Pride' mangoes during ripening (RP) at 21 ± 1°C without storage (0W), and after 3 and 5 weeks of storage (3W and 5W, respectively), as influenced by 1-MCP and CA (T). Vertical bar outside graphs represent LSD ($P \leq 0.05$). Vertical bars within graphs represent SEM, n = 3 replications. LSD ($P \leq 0.05$) for *endo*-PG: T = 0.86, RP = 1.49, T x RP = 2.11; *exo*-PG: T = ns, RP = 11.15, T x RP = ns. PE: T = ns, RP = 0.07, T x RP = ns. RP = ripening period, W = weeks of storage, D = days of ripening period.

7.3.2.3. Fruit skin colour and pigments

The subjective colour measurement was conducted during ripening of 'Kensington Pride' mango following 3 and 5 weeks of CA storage which is presented in Figure 7.11. The colour of control fruit was scored between 4 and 5 which was classified as 75 – 100% yellow but 1-MCP treated fruit without storage only developed about 50% yellow in skin colour after nine days in shelf. The colour development was suppressed in mango fruit retrieved from CA storage after 3 and 5 weeks, and through the ripening period of both storage intervals, irrespective of 1-MCP treatment. The colour of fruit at the retrieval from three weeks of CA storage was scored 1.25 and 1.38 in 1-MCP-untreated and treated fruit which was not significantly different from five weeks of CA storage. After ripening period on the shelf, the yellow colour was developed about 50% in CA-stored fruit without 1-MCP treatment whereas 25% in 1-MCP-treated fruit. The prolonged storage duration effectively retarded the colour development in mango. No significant changes in colour of CA-stored fruit after five weeks of storage and the following ripening period.

The colour of the ripe mango fruit was significantly ($P \leq 0.05$) influenced by 1-MCP treatment, CA storage duration, and the interaction between them. The retention of green colour by 1-MCP treatment in ripe mango skin was depicted by lower a* value compared to untreated fruit; and it was accompanied by lower hue° and higher chroma of ripe fruit (Figure 7.12). During ripening, the b* value in all fruit showed a significant increase, but minor increment was exhibited in 1-MCP treated fruit resulted in lower b* value compared to untreated fruit.

During fruit ripening, the chlorophylls in untreated fruit decreased to the greater extent (60 and 46% after 3 and 5 weeks of storage, respectively) compared to 1-MCP treatment which maintained the reduction in chlorophylls content at lower rate (45 and 23% after 3 and 5 weeks of storage intervals, respectively), resulted in higher chlorophylls concentration in ripe 1-MCP-treated after 5 weeks CA storage (Figure 7.13). Besides the retention in chlorophylls content, 1-MCP treatment also reduced the concentration of carotenoids in mango skin compared to control during normal ripening. Similar reduction trend in carotenoids content was monitored during ripening of 1-MCP-treated fruit following 3 weeks of CA storage whilst no significant change was noticed in untreated fruit. However, this trend was in reverse

when the storage duration was prolonged to 5 weeks where 1-MCP treatment managed to retain its carotenoids content during ripening, consequently contained higher carotenoids compared to CA storage without 1-MCP treatment.

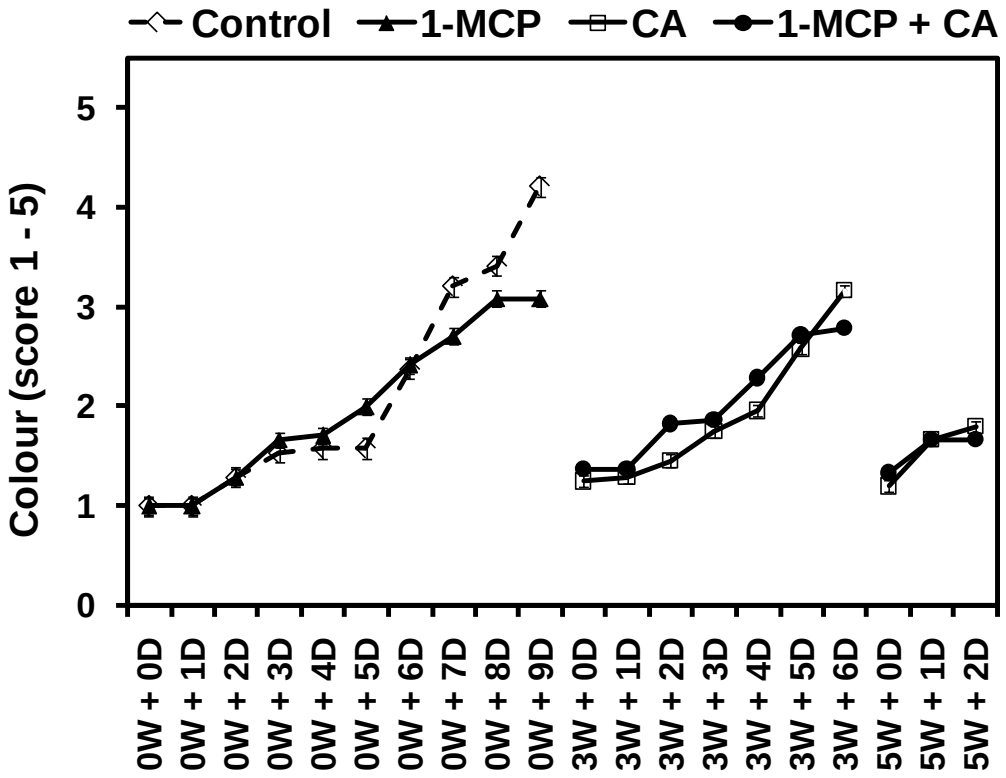

Figure 7.11. Changes in fruit skin colour of 'Kensington Pride' mangoes during ripening (RP) at 21 ± 1°C without storage (0W), and after 3 and 5 weeks of storage (3W and 5W, respectively), as influenced by 1-MCP and CA (T). Vertical bars within graphs represent SEM (n = 15; 5 fruit x 3 replications). LSD ($P \leq 0.05$) for colour at 0W: T = ns, RP = 0.25, T x RP = ns; 3W: T = ns, RP = 0.21, T x RP = ns; 5W: T = ns, RP = ns, T x RP = ns. RP = ripening period, W = weeks of storage; D = days of ripening period.

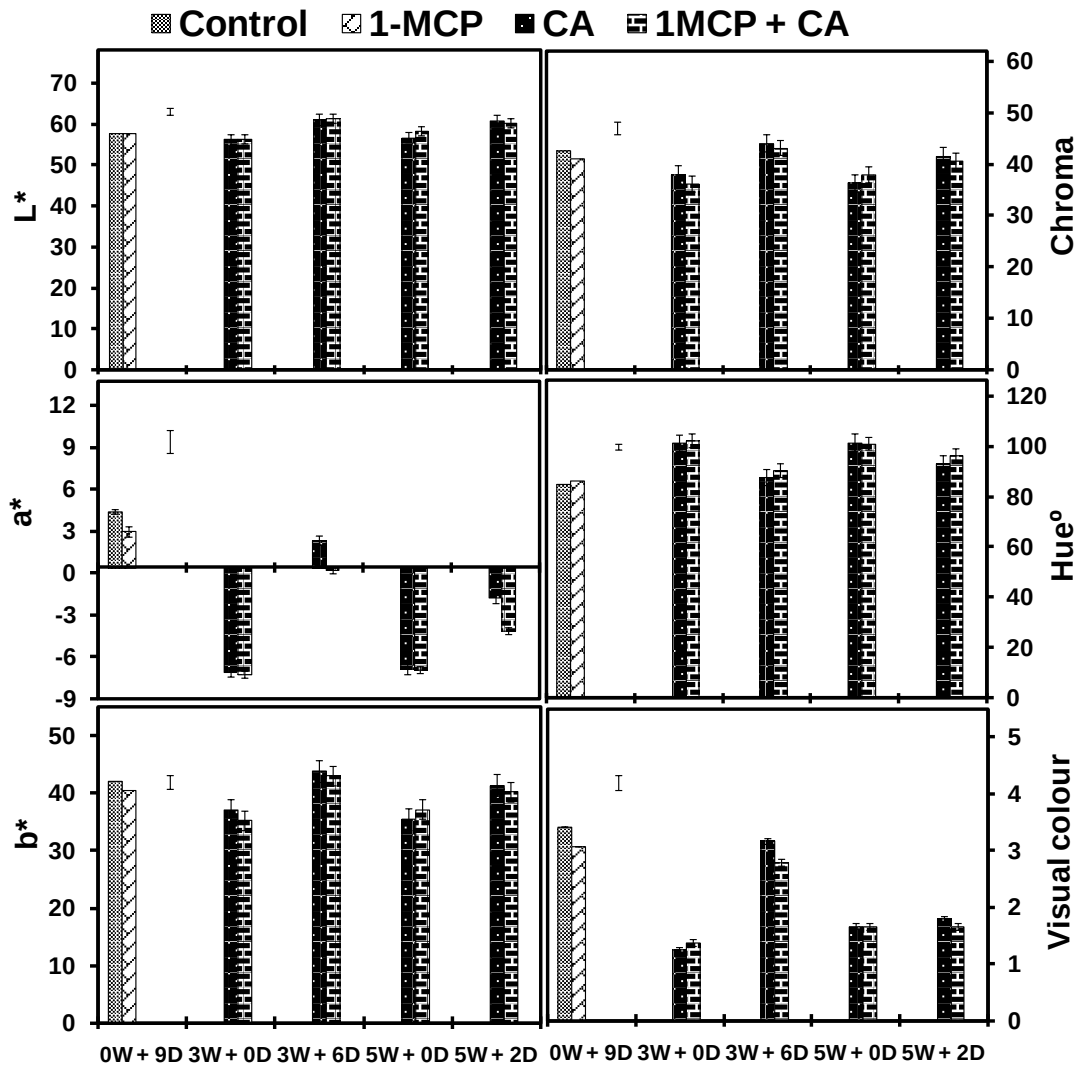

Figure 7.12. Changes in fruit skin colour of ripe 'Kensington Pride' mangoes at 21 ± 1°C without storage (0W), and after 3 and 5 weeks of storage (3W and 5W, respectively), as influenced by 1-MCP and CA (T). Vertical bars outside graphs represent LSD ($P \leq 0.05$). Vertical bars within graphs represent SEM (n = 15; 5 fruit x 3 replications). LSD ($P \leq 0.05$) for L*: T = 0.66, RP = 1.14, T x RP = 1.61; a*: T = 0.67, RP = 1.17, T x RP = 1.65; b*: T = 1.03, RP = 1.78, T x RP = 2.51; Chroma = T = 1.01, RP = 1.75, T x RP = 2.48; Hue°: T = 0.97, RP = 1.67, T x RP = 2.37; Visual colour: T = 0.11, RP = 0.19, T x RP = 0.27. RP = ripening period, W = weeks of storage, D = days of ripening period.

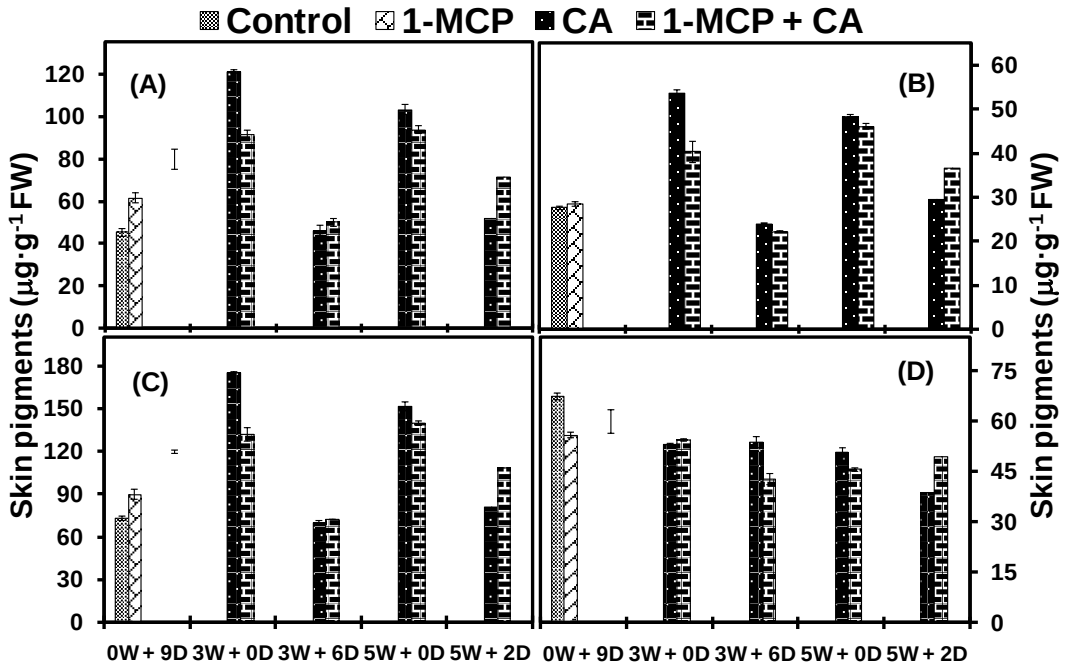

Figure 7.13. Changes in (A) chlorophyll a, (B) chlorophyll b, (C) total chlorophylls, and (D) carotenoids content of ripe 'Kensington Pride' mangoes at 21 ± 1°C without storage (0W), and after 3 and 5 weeks of storage (3W and 5W, respectively), as influenced by 1-MCP and CA (T). Vertical bars outside graphs represent LSD ($P \leq 0.05$). Vertical bars within graphs represent SEM, n = 3 replications. LSD ($P \leq 0.05$) for chlorophyll a: T = 3.83, RP = 6.63, T x RP = 9.37; chlorophyll b: T = ns, RP = 4.16, T x RP = ns; total chlorophylls: T = 5.78, RP = 10.01, T x RP = 14.15; carotenoids: T = ns, RP = 4.84, T x RP = 6.84. RP = ripening period, W = weeks of storage, D = days of ripening period

7.3.2.4. Sugars and organic acids

7.3.2.4.1. *Sugars*

Sucrose, fructose and total sugars concentration in ripe mango pulp was significantly ($P \leq 0.05$) influenced by ripening period but not by treatments (Figure 7.14). Glucose was significantly ($P \leq 0.05$) influenced by treatment, storage period, and their interaction. Glucose was detected only in 1-MCP treated fruit after 5 weeks of CA storage which was 4.55 $\mu g \cdot g^{-1}$ FW and the concentration decreased to 1.54 $\mu g \cdot g^{-1}$ FW during ripening. Sucrose and fructose concentration increased significantly during ripening of CA-stored mango fruit following 3 weeks of storage, and higher concentration was exhibited in 1-MCP-treated fruit compared to untreated one. However, after five weeks of storage the sucrose concentration in 1-MCP treated fruit was lower than its counterpart and no further change during ripening

period. Since the major sugar in mango fruit was sucrose, the changes in total sugars followed the trend in sucrose concentration (Figure 7.14).

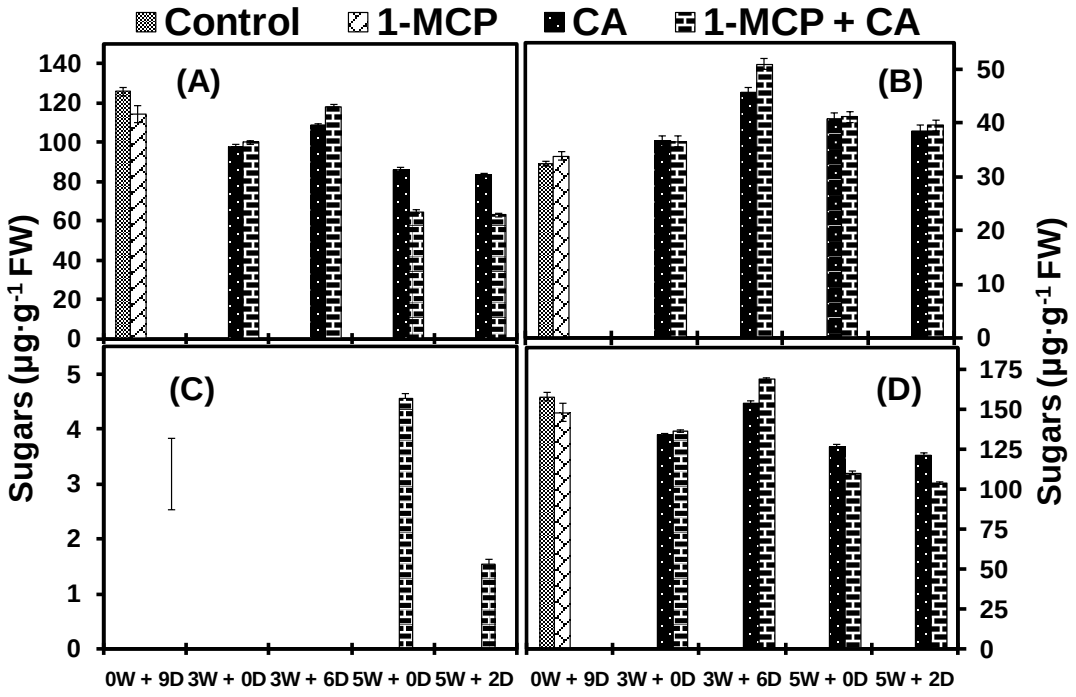

Figure 7.14. Changes in (A) sucrose, (B) fructose, (C) glucose and (D) total sugars of ripe 'Kensington Pride' mangoes at 21 ± 1°C without storage (0W), and after 3 and 5 weeks of storage (3W and 5W, respectively), as influenced by 1-MCP and CA (T). Vertical bars outside graphs represent LSD ($P \leq 0.05$). Vertical bars within graphs represent SEM, n = 3 replications. LSD ($P \leq 0.05$) for sucrose: T = ns, RP = 9.92, T x RP = ns; fructose: T = ns, RP = 3.07, T x RP = ns; glucose: T = 0.53, RP = 0.92, T x RP = 1.30; total sugars: T = ns, RP = 11.56, T x RP = ns. RP = ripening period, W = weeks of storage, D = days of ripening period.

7.3.2.4.2. *Organic acids*

The changes in organic acids level in 'Kensington Pride' mango pulp are presented in figure 7.15. The 1-MCP treatment did not significantly affect the organic acids level during ripening of CA-stored mango fruit. Significant changes were evaluated in citric and succinic acids during ripening of 3-week-stored fruit, but it remained stable during ripening after five weeks of storage. Citric acid reduced to a greater extent compared to succinid and malic acids, specifically in CA-stored fruit without 1-MCP treatment. On the other hand, succinic acid content in 1-MCP treated fruit increased while that in untreated fruit decreased during ripening

following three weeks of CA storage. After five weeks of CA storage, higher citric and succinic acids content was evaluated in 1-MCP-treated fruit compared to untreated one, and it remained higher during ripening although they were not statistically different. Malic acid was found in lesser amount and it was not statistically influenced by 1-MCP treatment or ripening period. Succinic acid was the predominant acid and its changes in concentration influenced the trend of changes of total organic acids in mango pulp.

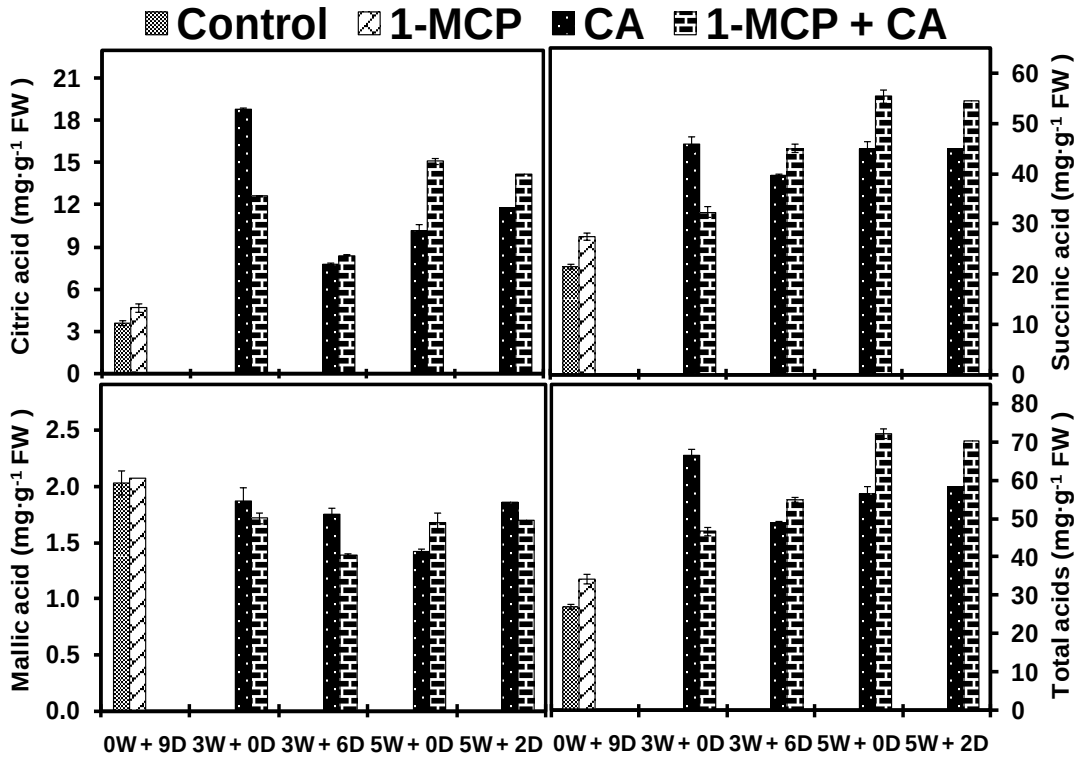

Figure 7.15. Changes in citric, succinic, malic and total acids of ripe 'Kensington Pride' mangoes at 21 ± 1°C without storage (0W), and after 3 and 5 weeks of storage (3W and 5W, respectively), as influenced by 1-MCP and CA (T). Vertical bars within graphs represent SEM, n = 3 replications. LSD ($P \leq 0.05$) for citric acid: T = ns, RP = 4.08, T x RP = ns; succinic acid: T = ns, RP = 7.59, T x RP = ns; malic acid: T = ns, RP = ns, T x RP = ns; total acids: T = ns, RP = 11.50, T x RP = ns. RP = ripening period, W = weeks of storage, D = days of ripening period.

7.3.2.5. Carotenoids, ascorbic acid and total antioxidants

Carotenoids, ascorbic acid, and total antioxidants in mango pulp changed significantly during ripening following CA storage intervals without significant influence of 1-MCP treatment (Figure 7.16). The significant effect of ripening period, and the interaction between ripening and 1-MCP treatment on the concentration of carotenoids had been measured in all fruit. Higher concentration of carotenoids was noted in 1-MCP-treated fruit compared to untreated one after 3 weeks in CA storage; and this was declined 1.7 fold in ripe 1-MCP-treated fruit while inclined 1.4 fold in untreated fruit. The decline in ascorbic acid concentration in 1-MCP-treated fruit was significantly lower than CA-stored without 1-MCP treatment, in which was accounted for 1.2 and 2.4 fold, respectively. No significant changes in carotenoids, ascorbic acids, and total antioxidants were measured during ripening of the 1-MCP-treated fruit after 5 weeks of CA storage. The changes in total antioxidants levels during ripening after each storage interval was in a similar pattern to ascorbic acid, suggesting that ascorbic acid contributed a significant effect on total antioxidants levels in mango pulp.

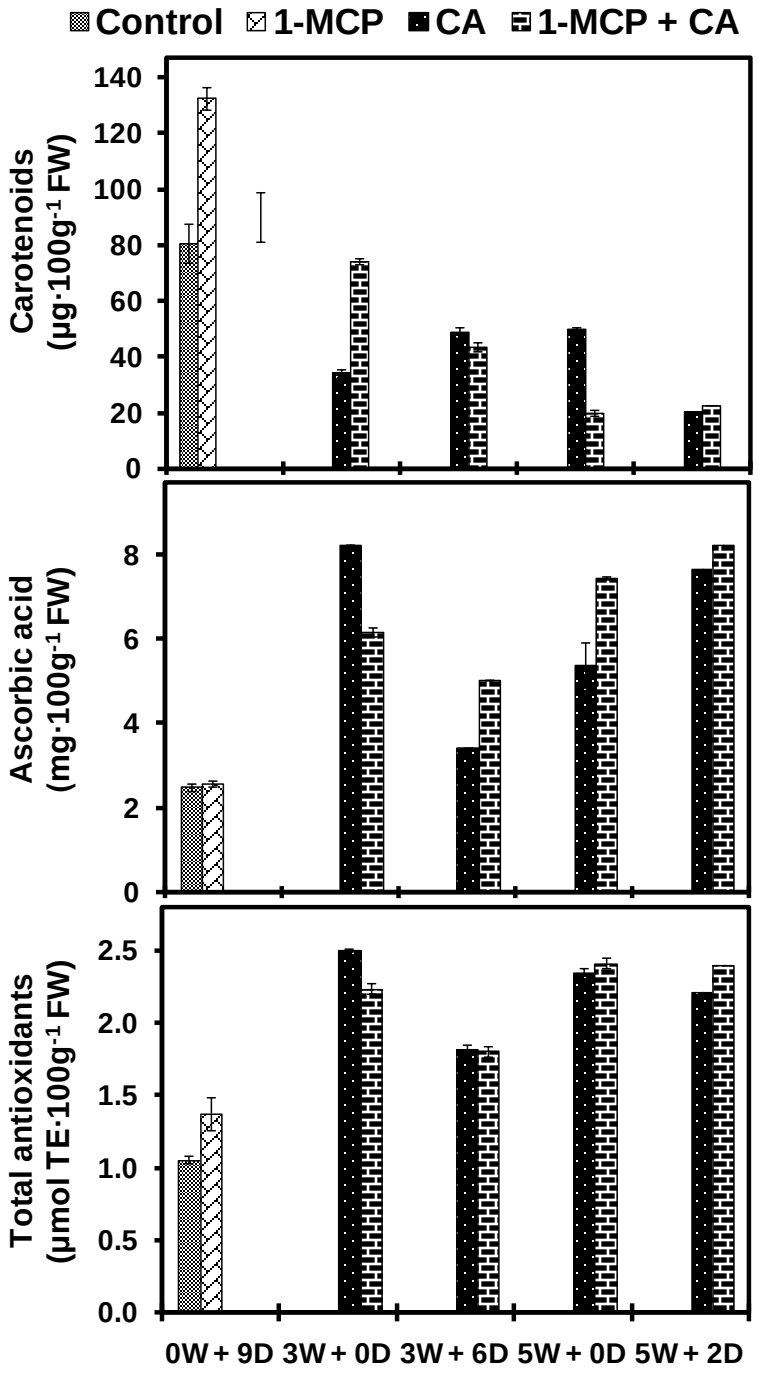

Figure 7.16. Changes in carotenoids, ascorbic acid, and total antioxidants of ripe 'Kensington Pride' mangoes at 21 ± 1°C without storage (0W), and after 3 and 5 weeks of storage (3W and 5W, respectively), as influenced by 1-MCP and CA. Vertical bars outside graphs represent LSD ($P \leq 0.05$). Vertical bars within graphs represent SEM, n = 3 replications. LSD ($P \leq 0.05$) for carotenoids: T = ns, RP = 12.40, T x RP = 17.54; ascorbic acid: T = ns, RP = 0.82, T x RP = ns; total antioxidants: T = ns, RP = 0.20, T x RP = ns. RP = ripening period, W = weeks of storage, D = days of ripening period.

7.4. Discussion

7.4.1. Fruit texture and softening enzymes activities

The 'Kensington Pride' mango stored in CA for 3 weeks maintained its firmness hence reduced fruit softening better than those stored in NA; however the firmness decreased as the storage proceeds to 5 weeks (Table 7.1). The softness of fruit stored in CA was significantly lower than fruit stored in NA for 3 and 5 weeks, irrespective of 1-MCP treatment (Figure 7.1) as has been reported by Lalel et al. (2003a) suggesting that 1-MCP had little impact on fruit softness during 3 or 5 weeks storage. The 1-MCP treatment combined with CA storage (10% CO_2) has been reported to effectively maintain fruit firmness in hot water-treated mango compared to lower CO_2 concentration (Sivakumar et al., 2012), suggesting that alteration in CO_2 composition during storage is highly influencing mango fruit firmness.

The composition of CA storage in this research was 3% O_2 + 5% CO_2 which may not be sufficient to have such effect when combined with 1-MCP. The decrease in fruit firmness is an indication of the ripening process which took place during storage (Noomhorm and Tiasuwan, 1995) due to the activity of softening enzymes (Singh and Singh, 2012; Yashoda et al., 2007). The pectolytic enzyme activities were depressed during 3 weeks of storage in all fruit (Figure 7.2) but an increase in PG activities was recorded in control fruit after 5 weeks of storage in NA at 13 ± 0.5°C. The retardation of *endo*-PG activity was more pronounced in CA-stored fruit without 1-MCP treatment compared to 1-MCP-treated fruit stored in NA and CA for 5 weeks. It has been reported previously that CA storage retards the degradation of pectic substances in 'Palmer' mango (Teixeira and Durigan, 2011) which may be linked to the depression on *endo*-PG activities in this results. Fruit treated with 1-MCP exhibited retardation on *endo*-PG activity after 5 weeks of storage with no significant difference from the combined effect of 1-MCP and CA storage; and it was not better than CA storage alone. A reduction in PG enzymes activities has been reported in 1-MCP-treated 'Tegan Blue' plum during cold storage (Khan and Singh, 2009).

The CA-stored mango was ripened in NA at 21 ± 1°C following 3 and 5 weeks of storage intervals. Mango fruit kept on the shelf at ambient temperature took 9 days for ripening whilst CA-stored fruit with the storage duration of 3 and 5

weeks required 6 and 2 days to ripe, respectively. After 3 weeks of storage, the 1-MCP treatment exhibited a significant higher inclining rate in softness within 3-5 days of ripening compared to untreated fruit; however it was maintained at the same level after 5 to 6 days. As the softness increase, the fruit firmness declined significantly during ripening following the same storage duration regardless of 1-MCP treatment. The 1-MCP treatment seemed to have an effect on the reduction rate of firmness during ripening, although it was not statistically significant. Delayed in fruit softening due to 1-MCP treatment has been reported in mango fruit (Bomfim et al., 2011; Lalel et al., 2003f). The synergistic effect of 1-MCP and CA storage on the reduction of fruit softening has been reported by Sivakumar et al. (2012) in hot-water-treated 'Kent' mango. The effect of 1-MCP combined with CA storage on ripe fruit firmness may be linked to the activities of PG and PE. The *endo*- and *exo*-PG activities were significantly increased during ripening after 3 weeks of CA storage; and 1-MCP treatment showed significant effect on *endo*-PG activity (Figure 7.10). The different inhibition effect of 1-MCP on PG and PE activities has been reported as a cultivar dependent (Singh and Neelam, 2008), and perhaps different storage condition, the concentration of 1-MCP and application techniques (Watkins, 2008) may have affected the effectiveness of 1-MCP treatment during ripening which warrants further investigations.

7.4.2. Fruit skin colour and pigments

During storage, 1-MCP treatment improved the skin colour of NA stored fruit, but when 1-MCP treatment was combined with CA storage the green colour was retained as depicted in low a* and high hue°. CA storage contributed to preserving the green colour during storage which may be due to the suppression of ripening process hence reduced the decomposition of chlorophylls (McLauchlan and Barker, 1992). However, the effect of 1-MCP in retarding the skin colour development during NA storage (Penchaiya et al., 2006) was not exhibited in this research which may be due to the different cultivars that may respond differently as has been reported (Singh and Singh, 2012).

The ripening process was terminated when the fruit firmness reached its eating soft value, and that was at 9, 6 and 2 days following the 0, 3, and 5 weeks of storage intervals. Although the softness was scored at eating soft, the yellow colour

was not improved in 1-MCP-treated fruit kept on shelf and during ripening after CA storage (Figure 7.11). The green colour was persisted after CA storage and significantly lower a* was evaluated in 1-MCP treated fruit as compared to its counterpart (Figure 7.12). However, 1-MCP treatment did not show any influence in skin pigments but ripening period did. An interaction effect between 1-MCP treatment and ripening period was noticed in carotenoids concentration. During ripening, 1-MCP-treated fruit stored for 3 weeks accumulated lower carotenoids while those stored for 5 weeks in CA kept higher carotenoids compared to untreated fruit (Figure 7.13) while its level was maintained similar to the level measured directly after 5 weeks storage. 1-MCP has been reported to delay chlorophylls degradation (Jiang and Joyce, 2000) but increase the β-carotenoid content (Islas-Osuna et al., 2010) during storage of mango fruit. The combined effect of 1-MCP and CA storage may have effect on the preservation of the chlorophylls and carotenoids content in 'Kensington Pride' mango.

7.4.3. Sugars and organic acids composition

The 1-MCP-treated fruit accumulated sugars during 3 weeks of storage, regardless the atmosphere composition; however, the accumulation of organic acids was noticed after 5 weeks of storage in 1-MCP treated and stored in CA. TSS and TA value of 1-MCP-treated 'Kent' mango was not significantly different from untreated fruit during NA storage (Islas-Osuna et al., 2010) but the ratio increased during CA storage (Sivakumar et al., 2012) suggested that total sugars content is higher than acids content. An increase in total acids during CA storage of 1-MCP-treated 'Kensington Pride' mango may be due to O_2 (3%) composition in this research which was lower compared to previous report. The synthesis of sugars and organic acids during ripening was not affected by 1-MCP treatment; however the significant changes were due to the storage duration and ripening period. The fruit accumulated sugars during ripening of 3-week-stored fruit but it was maintained at the same level during ripening of 5-week-stored counterpart. In contrast, 1-MCP treated fruit managed its acids concentration at high level after 5 weeks of storage although it was not significantly different from the control fruit. A reduction in sugars and preserving high organic acids concentrations during ripening of 1-MCP treated 'Kensington Pride' mango fruit has been reported by Razzaq et al., 2015. The concentration of sugars has been reported to increase during normal ripening of

'Keitt' mango while acids reduced (Medlicott and Thompson, 1985). My findings suggest that the ripening process may be hampered by the combination effect of CA after 5 weeks of storage that inhibited the respiration and C_2H_4 production and 1-MCP which has been reported to delay the ethylene production (Singh and Singh, 2012).

7.4.4. Nutritional quality

1-MCP treatment has been reported to retain β-carotene and ascorbic acid during storage of mango fruit (Islas-Osuna et al., 2010). 1-MCP treatment has also been reported to retain higher ascorbic acid and antioxidant activity in hot-water-treated mango fruit during CA storage composed of 3% O_2 and 8% CO_2 compared to 5% O_2 and 5% CO_2 (Sivakumar et al., 2012). During storage, 1-MCP without CA maintained carotenoids but in CA reduced while ascorbic acids and total antioxidants were maintained during 5 weeks of storage. In this research, however, the carotenoids content of 1-MCP-treated fruit stored for 3 weeks under CA decreased significantly during ripening and it was maintained at its post-storage level after 2 days ripening following 5 weeks of storage. Ascorbic acid and total antioxidants in 1-MCP-treated fruit were reduced during ripening following 3 weeks of storage but it was maintained at high level in ripe fruit following 5 weeks of storage (Figure 7.16). Cultivar, atmosphere composition and ripening condition may be contributed to the differences in nutritional composition reported previously and in this research.

In conclusion, 1-MCP treatment together with CA induced pulp carotenoids synthesis and sugars to acids ratio within three weeks storage. However, it did not affect fruit softening and its enzyme activities, skin colour and pigments, total sugars, and total antioxidants during three or five weeks storage. Prolong storage to five weeks resulted in increasing organic acids concentration. Fruit stored for 3 weeks reach its fully ripe stage after 6 days whilst that for 5 weeks only 2 days in shelf. The combination effect of pre-storage treatment of 1-MCP and CA storage (3% O_2 + 5% CO_2) resembled to CA storage alone in overall quality of fruit during ripening following three weeks storage. Prolonged storage to five weeks resulted in low quality of ripe mango fruit.

Chapter 8

General discussion, conclusions, and future research

8.1. Introduction

Controlled atmosphere (CA) storage has been reported to prolong the storage life and maintain the quality of mangoes. However, there are several issues regarding physiological and biochemical alterations during CA storage which adversely affect the fruit quality. The critical factor during storage is the composition of oxygen (O_2) and carbon dioxide (CO_2) since a minor variation from its optimum requirement may result into a stress response that contributes to the development of poor fruit quality (Kader, 2003a). The optimal CA condition varies upon cultivar, maturity stage, temperature and exposure time (Brecht et al., 2003; Singh and Singh, 2012). Earlier studies on CA storage of mango fruit were focused on extending the postharvest life and maintaining its flavour and aroma quality (Lalel and Singh, 2004; Lalel et al., 2001; Lalel et al., 2006, Singh and Zaharah, 2015) while other quality parameters have not been studied in details. The traditional CA storage usually deploys with low temperature to prolong the storage life by reducing the respiration and C_2H_4 synthesis (Singh and Singh, 2012; Thompson, 1998).

Recently, the postharvest application of plant hormone such as methyl jasmonate (MJ) (Czapski and Saniewski, 1992; Gonzalez-Aguilar et al., 2001; Lalel et al., 2003g; Saniewski et al., 1987; Tasneem et al., 2004), putrescine (Put) (Khan and Singh, 2007b; Malik and Singh, 2006; Martínez-Romero et al., 2002; Perez-Vicente et al., 2001; Tasneem et al., 2004), and a non-hormonal compound, 1-methylcyclopropane (1-MCP) (Hofman et al., 2001; Hojo et al., 2006; Jiang and Joyce, 2000; Lalel et al., 2003f; Pandey and Singh, 2007; Santos et al., 2004; Singh, 2011; Wang et al., 2006) has been extensively studied to regulate the ripening process and improve the quality of climacteric fruit including mango. Efforts have been made to improve the effectiveness of low O_2/high CO_2 condition at low temperature management in controlling the fruit quality during storage and shelf-life by pre-storage treatment which involves the application of C_2H_4 precursor or inhibitor (Asif et al., 2009; Gonzalez-Aguilar et al., 2003; Siripatrawan and

Assatarakul, 2009; Sivakumar et al., 2012). However, there were gaps of information regarding those synergistic effects in regulating the quality of 'Kensington Pride' mango, and that was the general objective of this study.

8.2. Controlled atmosphere extends the storage life and maintains the quality of 'Kensington Pride' mango during ripening

8.2.1. Regulation of fruit softening and colour degradation in CA-stored fruit

Fruit ripening is a postharvest phenomenon in mango within a short period of time at ambient temperature (Gomez-Lim, 1997). The most intrigue changes during this postharvest period are in firmness and colour which directly affect the consumer's acceptance. Controlled atmosphere storage in association with low temperature has been set up to lessen the vast changes hence prolong the storage life and maintain the quality of mango fruit (Abdulah and Basiouny, 2000; Bender et al., 2000a; Bender et al., 2000b; Kader, 2003b; Kim et al., 2007; Lalel et al., 2005; Lalel et al., 2006; Lizana and Ochagavia, 1996; Maekawa, 1990; Noomhorm and Tiasuwan, 1995) including 'Kensington Pride' mango cultivar (Dang et al., 2008b; Lalel et al., 2001; Lalel et al., 2003c; McLauchlan and Barker, 1992). However, several problems such as tissue injury, fruit softening, poor colour development, and off-flavour in ripe mango are exhibited following CA storage (Anonymous, 2002; Beaudry, 1999; Bender et al., 2000a; Bender et al., 2000b; Dang et al., 2008b; Lalel et al., 2006; McLauchlan and Barker, 1992) which could be due to a slight shift in the storage atmosphere composition beyond the optimum requirement of the cultivar. The O_2 concentration between 1 and 10% has been reported to maintained mango fruit firmness (Teixeira and Durigan, 2011; Gonzalez-Aguilar et al., 1997; McLauchlan and Barker, 1992; Rao and Rao, 2008). In this study, 3% of O_2 was chosen since it was considered as the lowest concentration where the normal metabolism is temporary inhibited as has been reported by Beaudry (1999). The experimental result of this research showed CA storage comprised of 3% O_2 and 5% CO_2 maintained better fruit firmness (Figure 4.1) which may due to the inhibition of pectin esterase (PE) activity (Figure 4.3) within 4 weeks of storage duration. The inhibition of *exo*- and *endo*-PG activities was measured in all CA-stored fruit during 3 weeks of storage, suggested that longer storage duration may not effectively reduce these enzyme activities. This CA composition also retarded the degradation of

chlorophylls hence reduced the de-greening of mango skin during storage but concomitantly increased the carotenoids level (Figure 4.4 and 4.5). Delayed in fruit softening and colour degradation but increased carotenoids concentration during CA storage may be contributed by the reduction of the rate of ripening process as has been previously reported in mango fruit due to low O_2 and high CO_2 concentration (Batu and Thompson, 1998; Lalel et al., 2001; Lalel et al., 2005). Reduction in pectolytic enzymes activity may be accelerated by CO_2 concentration when it retards the hydrolysis of pectic substances (Kays and Paull, 2004; Wills et al., 1998) during low respiratory metabolism (Bender et al., 2000c; Watkins and Zhang, 1998).

CA storage did not affect the fruit softening and colour changes (Figure 4.11 and 4.14) during 4 days on shelf ripening. The reduction in fruit firmness during ripening of CA-stored fruit is in accordance to the previous report (Yahia and Hernandez, 1993) where under normal condition cell wall substances degrades by synergistic action of pectolytic enzymes (Selvaraj and Kumar, 1989; Singh et al., 2013; Singh and Singh, 2012). Higher *exo*-PG and PE than *endo*-PG activities (Figure 4.12 and 4.13) may be an indication of the active site of cell wall polymer hydrolysis which may be started by de-esterification and hydrolysis at the terminal end of the polymer chain (Abu-Sarra and Abu-Goukh, 1992; Lazan et al., 1986). Higher *exo*-PG activity compared to *endo*-PG has been reported in 'Nam Dok Mai' mango (Suntornwat et al., 2000) that highly related to ripening (Chaimanee, 1992). Improving in yellowness of skin colour was noticed during ripening of CA-stored fruit which is similar to previously reported in 'Delta R2E2' (Lalel et al., 2005) although carotenoids concentration in all fruit were depleted. However, the reduction in chlorophylls and increase in carotenoids concentration was evaluated during storage suggested that the ripening process may have been started during storage, although the rate in CA-stored fruit was lower compared to NA-stored ones. All these changes phenomena may suggest that the CA-stored fruit regained their normal metabolism (Chaimanee et al., 2000; Mathooko, 1996; Toivonen and Brummell, 2008; Yashoda et al., 2007) without a sign of tailing effects of CA storage (Lalel et al., 2001; Lalel et al., 2005) following 4 weeks of CA storage.

8.2.2. Effect of CA storage on sugars, organic acids, and bioactive compounds

The major sugar in green mature 'Kensington Pride' mango was fructose followed by sucrose while glucose was in trace amount, which similar to 'Irwin' mango cultivar reported by Ito et al. (1997). Increasing sugars accumulation to their peak was evaluated after 3 weeks of storage and remained higher compared to NA-stored fruit after 4 weeks of storage (Figure 4.6). Initially, Succinic was found as the major organic acid in this cultivar followed by citric and malic acid. The significant influence of CO_2 concentration was noticed in citric and succinic acid concentrations (Figure 4.7) hence increased the accumulation of acids during CA storage higher than NA. Bender et al. (2000c) reported that 'Haden' and 'Tommy Atkins' mangoes lost their starch content during 3 weeks of CA storage. In other report, the total soluble solids and sugars content in 'Alphonso' and 'Banganapali' mangoes increased within 4 weeks under similar CA storage condition (Rao and Rao, 2008). In connection to my result, this may suggest that the 3% O_2 in storage did not affect the respiratory oxidation of carbohydrate but 4 – 6% CO_2 might exhibit an inhibition effect on the oxidation of TCA cycle intermediates (Mir and Beaudry, 2002). Concerning the sugars composition, fruit ripened normally with higher reducing sugars concentration (except those stored in 6% CO_2, Figure 4.16) compared to NA-stored fruit after 4 weeks of storage, and similar accumulation of sugars has been reported in 'DeltaR2E2' (Lalel et al., 2005). Decreasing amount of organic acids during ripening (Figure 4.17) may also indicate that CA storage has temporary inhibition effect on respiration and ripening process (Mir and Beaudry, 2002) and the process resumed its activity when fruit were stored in ambient condition.

CA condition suppressed the carotenoids and total antioxidants in mango pulp during 4 weeks of storage (Figure 4.8 and 4.10) but it was not affected the ascorbic acid content (Figure 4.9). It has been reported that β-carotene in 'Kensington Pride' mango increased as the CO_2 concentration increased during 5 weeks of storage (Lalel et al., 2001; Lalel et al., 2003a). The high rate of carotenoids synthesis was noticed during ripening although the accumulation was lower compared to NA-stored fruit after 4 days of ripening. The concentration of ascorbic acid was maintained at higher level in CA-stored fruit compared to NA-stored ones, showed that CA storage preserved the ascorbic acid concentration during storage and

ripening of 'Kensington Pride' mango. This result was in contrast to previous report by Tefera et al. (2007).

8.3. The role of methyl jasmonate (MJ) on fruit softening, colour development and quality during CA storage

Jasmonic acid and its volatile ester methyl jasmonate (MJ) are naturally occurring plant growth regulators which induce higher-plant responses including environmental stress (Creelman and Mullet, 1997), ripening (Fan et al., 1998b; Lalel et al., 2003g), and senescence (Ananieva et al., 2007). The exogenous application of MJ has been reported to increase the ethylene production of climacteric fruit (Fan et al., 1998) including 'Kensington Pride' mango (Lalel et al., 2003g) hence promotes the ripening process. The application of methyl jasmonate together with modified atmosphere packaging has improved the quality of papaya without off-flavour development (Gonzalez-Aguilar et al., 2003). During this study, the firmness of MJ-treated 'Kensington Pride' mango stored in NA showed similar reduction with untreated fruit, indicated that the fruit underwent ripening process as the nature of MJ was not affected. However, MJ-treated fruit stored in CA exhibited higher fruit firmness during storage (Table 5.1) compared to those fruit stored in NA which was affected by CA storage as has been reported by previous researchers (Abdulah and Basiouny, 2000; Kader, 1993; Lalel et al., 2003g, 2005; McLauchlan and Barker, 1992; Noomhorm and Tiasuwan, 1995). The retardation of fruit firmness followed by the reduction of pectolytic enzyme was evaluated during 2 to 4 weeks of storage (Table 5.2 and Figure 5.2). Compared to CA storage alone, higher rate of softening in MJ-treated fruit during CA storage compared to CA storage alone suggested that MJ may have lessened the CA effect as has been previously reported in apple (Argenta et al., 2010). The fruit firmness was positively correlated to *endo*-PG and PE (Table 5.3) where fruit firmness declined after 2 weeks of storage with reduction in those enzyme activities.

Mango fruit treated with MJ showed the reduction in skin green colour as well as chlorophylls content and concomitantly improved carotenoids concentration after 4 weeks of low temperature storage in NA. Reduction in skin green colour was also monitored in all fruit stored in CA but in lower rate compared to NA stored ones. However, the carotenoids content in MJ-treated fruit stored in CA was

preserved within longer period which was not exhibited in other fruit (Figure 5.1). It has been reported that disappearance of the chlorophylls in JA-treated tomato and chlorosis symptoms in leaves is associated to the increasing ethylene-forming enzyme (Saniewski et al., 1987) and the signal of senescence (Creelman and Mullet, 1997). Sugars were accumulated in MJ-treated fruit during 6 weeks of CA storage with sucrose as the predominant sugars (Figure 5.2) while organic acids was reduced within 2 weeks of storage (Figure 5.3) which may increase the sugars to acids ratio hence may affect the taste and flavour of fruit. Higher ratio of sucrose to fructose was exhibited in MJ-treated fruit stored in CA compared to that in NA after 6 weeks of storage. It has been suggested that cultivars and the degree of sugar metabolism are factors that affect the sugars proportion in fruit (Singh and Singh, 2012). The suppression on carotenoids, ascorbic acid and total antioxidants levels in mango pulp was measured in MJ-treated fruit stored in CA. The concentration of CO_2 (5%) during storage may be the factor that affected the carotenoids accumulation and it was not alleviated by pre-storage treatment of MJ. It has been reported that 'Kensington Pride' mango accumulates higher β-carotene during storage under 9% CO_2 compared to those stored in lower CO_2 concentration (Lalel et al., 2001). However, carotenoids content in mango pulp was increased in MJ-treated fruit stored in NA and this is in accordance to previous research (Perez et al., 1993; Saniewski et al., 2010). In general, the postharvest application of MJ combined with CA storage resulted in better fruit quality during 4 weeks of storage at 13°C compared to their lone action which may due to their opposing effects on ripening process.

8.4. The role of putrescine on fruit softening and colour development during CA storage

Putrescine (Put) is one of the polyamines known as plant regulator which involves in regulating ranges of biological process including ripening, senescence, secondary metabolites synthesis, enzymes activity and responses to stress (Bouchereau et al., 1999; Fariduddin et al., 2013; Kussano et al., 2008; Malik, 2003). Reduction in fruit firmness was exhibited in all fruit during 2 weeks of storage. However, after 4 weeks of storage, the application of Put prior to CA storage resulted in slightly better retention in fruit firmness than CA storage alone. Previously the action of Put on fruit firmness has been reported in mango (Singh et al., 2013) and

plum (Khan and Singh, 2007b) during cold storage. The lower reduction in fruit softening of Put-treated mango during 4 weeks of CA storage suggested that the ripening process may have been delayed hence improved the storage life. In conjunction to the reduction in softening, the *exo*-PG activity was increased in all fruit after 4 weeks of storage and reduced throughout 6 weeks (Figure 6.1.A), although it was measured higher in Put-treated fruit stored in CA compared to others. In contrary, all fruit exhibited reduction in *endo*-PG activity within 2 (Put-treated and CA-stored fruit) and 4 (Put-treated stored in CA and NA-stored fruit) weeks of storage (Figure 6.1.B). However, an unexpected increase in *endo*-PG activity was measured in Put-treated fruit stored in CA for 6 weeks which may demonstrate the action of Put in overcoming stress under prolonged CA storage. PE activity declined in all fruit after 2 weeks of storage (Figure 6.2). The effectiveness of Put treatment in preventing fruit softening and its enzyme activities is after 3 weeks in low temperature storage as has been reported in plums (Khan and Singh, 2007b). The influence of Put treatment on *endo*-, *exo*-PG and PE activities during low temperature storage in peach has been reported by Meng et al. (2009) which is in similar trend to my result. Firmness showed a negative correlation with *exo*-PG activity but a positive one with *endo*-PG and PE (Table 6.3).

The fruit skin colour turned yellow during storage in normal air while CA-stored fruit retained the green colour during 6 weeks of storage, regardless Put treatment (Figure 6.3). The chlorophylls content in all fruit declined at different rate during storage where lower reduction was measured in CA-stored fruit compared to NA-stored fruit. The carotenoids concentration, however, was maintained at its initial level in CA-stored fruit while it was increased in its counterpart at different storage duration. All Put-treated fruit lost the skin carotenoids content at 6 weeks of storage while the untreated fruit stored in CA and NA after 4 weeks of storage (Figure 6.4), and this may reflect the ability of Put treatment in prolonging the storage life of mango fruit. The delay in colour development as the effect of Put treatment has been reported in 'Delta R2E2' (Lalel et al., 2005) and 'Kensington Pride' mango (Malik and Singh, 2006) during low temperature storage.

Reducing sugars concentration increased in all fruit during 6 weeks of storage with different rates which may explicitly show the carbohydrate metabolism while storage condition may in effect on its rate. The highest accumulation of sugars and

organic acids was measured in Put-treated fruit stored for 6 weeks under CA condition (Figure 6.5 and 6.6) and this resulted in higher sugars to acids ratio compared to others, except control fruit. All fruit showed increasing trend in carotenoids concentration but CA-stored fruit showed very low accumulation during 6 weeks of storage. However, the ascorbic acid and total antioxidants were reduced in all fruit. Lower rate of reduction in ascorbic acid was measured in untreated fruit compared to Put-treated fruit, regardless the storage condition. The change in ascorbic acid may have contributed to the change in total antioxidants where the lowest level was measured in Put-treated fruit stored under normal air (Figure 6.7). Increasing in sugars and total soluble solids concentration while reducing in acids including ascorbic acid content in ripe 'Kensington Pride' mango with the pre-harvest treatment of Put has been reported earlier by Malik and Singh (2006). The ripening process may have not been altered during 6 weeks of storage when mango treated with Put and subsequently stored in CA.

8.5. The role of 1-MCP treatment combined with CA storage on mango fruit quality

The reduction in fruit softening and inhibition of fruit skin colour development occur during CA storage of 'Kensington Pride' mango, irrespective of the pre-storage treatments (Chapter 4, 5, 6). The reduction of fruit firmness in mango fruit has been reported to be in association with fruit ripening (Singh and Singh, 2012), and this may suggest that the ripening occurs under slow progression during CA storage. The lower fruit softness was also measured in CA-stored fruit during 5 weeks of storage (Figure 7.1) but the pulp firmness was at the same level as other treatments after 5 weeks of storage (Table 7.1). It has been reported that 1-MCP combined with CA (10% CO_2) storage is effectively maintained hot water-treated mango (Sivakumar et al., 2012). The different effect may be due to the variation in CO_2 composition where 5% of CO_2 was used in this present study. The activities of softening enzymes, PG and PE, were depressed during 3 weeks of storage in all fruit (Figure 7.2), while more pronounced effect of CA storage to retard the *endo*-PG activity was measured after 5 weeks of storage. CA storage retards the degradation of pectic substances in 'Palmer' mango (Teixeira and Durigan, 2011) suggesting that the pectolytic enzymes activity is also retarded which may have a link to the inhibition on the *endo*-PG activity in this study. The fruit treated with 1-

MCP exhibited an improvement in fruit skin colour during NA storage and it did not show any effects on colour when fruit stored in CA (Figure 7.3). Preserving green colour may be due to the delay in ripening process hence reduced the degradation of chlorophylls content (Figure 7.4) which has been reported as the effect of low O_2 and high CO_2 during storage of 'Kesington Pride' mango (McLauchlan and Barker, 1992). The effect of 1-MCP to retard the skin colour development as has been reported by Penchaiya et al. (2006) is not at the similar track as the result in this research which may be due to cultivar variations (Singh and Singh, 2012). Sugars and acids concentration was increased in 1-MCP-treated fruit after 3 and 5 weeks of CA storage, respectively (Figure 7.5 and 7.6). Sivakumar et al. (2012) reported that 1-MCP treatment combined with CA increased the ratio of TSS and TA in 'Kent' mango fruit, suggesting that the sugars content is higher than acids accumulation during storage. Within 3 weeks of CA storage, all fruit showed decreasing ascorbic acid and total antioxidants but increasing carotenoids content by different rates. During 5 weeks in CA storage, 1-MCP treatment maintained the bioactive compounds, except carotenoids, levels in mango pulp (Figure 7.7). It has been reported that 1-MCP treatment retains β-carotene and ascorbic acid in mango (Islas-Osuna et al., 2010). The combined effect of 1-MCP treatment and CA storage (3% O2 + 8% CO_2) has also reported to retain higher ascorbic acid and antioxidant activity compared to those stored in (5% O_2 + 5% CO_2) (Sivakumar et al., 2012), and this may have caused different result in the present study.

CA-stored fruit reached the eating soft stage within 5 and 2 days following 3 and 5 weeks of storage, respectively while those on shelf without storage took 9 days (Figure.7.8). 1-MCP treatment has been reported to delay mango fruit softening during low temperature storage (Bomfim et al., 2011; Lalel et al., 2003f; Singh et al., 2007). In this research, the 1-MCP-treated fruit stored in CA showed lower firmness in its ripe fruit compared to fruit without storage, however it maintained the firmness better than the untreated CA-stored fruit (Figure 7.9). The synergistic effect of 1-MCP and CA has been reported to reduce the softening in the hot-water-treated mango (Sivakumar et al., 2012). The linkage between the changes in fruit softening and the pectolytic enzymes activity may be depicted in the increasing activity of *endo*- and *exo*-PG during ripening following 3 weeks of CA storage; and 1-MCP treatment showed a significant influence on *endo*-PG activity (Figure 7.10). Higher

dose of 1-MCP treatment (1.0 $mg \cdot l^{-1}$) has been reported to delay the softening-related enzymes activity in 'Chausa', 'Langra', and 'Dashehari' mangoes (Singh and Neelam, 2008) whereas in this research, 0.6 $\mu l \cdot l^{-1}$ of 1-MCP was used. The different inhibitory effect of 1-MCP on PG and PE activities is a cultivar dependent as has been reported by Singh and Singh (2012) which may be argued as another factor that influence the effectiveness of 1-MCP treatment. Further influence of 1-MCP during ripening was noticed in the skin colour development. Although the eating soft stage was achieved, the yellowness of 1-MCP-treated fruit was not improved following any storage intervals (Figure 7.11). Lower a* value was evaluated in ripe 1-MCP-treated fruit compared to the untreated fruit (Figure 7.12). The skin green colour of 1-MCP-treated fruit on shelf was accompanied by higher chlorophylls and lower carotenoids content than control (Figure 7.13). During ripening of 1-MCP-treated fruit following 3 weeks of CA storage resulted in a decreasing carotenoids content, however those fruit retrieved after 5 weeks of CA storage was able to maintain the the carotenoids content at its post-storage level after 2 days ripening. Cultivars, dose of 1-MCP treatment, atmosphere composition and ripening condition may have some influence in the compositional changes during ripening which in part was different from previously reported. The accumulation of sugars was noticed in all 1-MCP-treated fruit after 3 weeks of storage (Figure 7.14) while organic acids increased after 5 weeks in 1-MCP-treated and stored under CA (Figure 7.15). It has been reported that TSS/TA ratio in hot-water-treated mango increased during CA storage (Sivakumar et al., 2012) may be similar to the increasing ratio of sugars and acids after 3 weeks of storage in my experiment. 1-MCP treatment did not affect sugars and organic acids accumulation during ripening but ripening time and storage duration did. 1-MCP-treated fruit stored for 3 weeks resulted in higher sugars to acids ratio at its ripe stage compare fruit stored for 5 weeks, and it may indicate that fruit underwent normal ripening after the 3 weeks of CA storage while during prolonged storage duration the ripening process may be hampered. Increasing in sugars concentration during normal ripening of mango fruit has been reported by Medlicott and Thompson (1985) while the inhibition of respiration and C_2H_4 production during CA storage and as the effect of 1-MCP treatment has been reported by Singh and Singh (2012) and Razzaq et al (2015). 1-MCP treatment together with 3 weeks of CA storage maintained the level of ascorbic acid and total

antioxidants but reduced carotenoids in ripe fruit; and it was in reverse during storage of 1-MCP-treated fruit under NA condition. The change in carotenoids was similar to ascorbic acid and total antioxidant levels during ripening at 21 ± 1°C (Figure 7.16). The composition of CA storage may have influenced the level of bioactive compounds in this research. The similar effect has been reported in hot-water-treated mango by Sivakumar et al. (2012).

8.6. Conclusions

Controlled atmosphere storage comprising of 3% O_2 and 5% CO_2 at 13°C delayed the ripening process for 4 weeks by maintaining fruit firmness, skin colour and pigments, sugars and acids ratio, and bioactive compounds such as carotenoids, ascorbic acids and total antioxidants level in mango fruit. Following four weeks of storage, the residual effect of CA storage was exhibited in skin colour development but it did not affect the ratio of sugars to acids, and the accumulation of bioactive compounds in ripe fruit. In combination with CA storage, the postharvest The pre-CA storage application of MJ reduced fruit firmness and suppressed the *exo*-PG activity, pusponed degreening as well as chlorophylls degradation, and increased sugars to acids ratio within four weeks of storage at 13°C. However, MJ treatment combined with CA storage did not increase carotenoids, ascorbic acid, and total antioxidants levels in mango fruit pulp throughout the storage period. Put treatment retained the fruit firmness and springiness, which negatively correlated to the *exo*-PG enzyme activity, and retarded the fruit degreening and chlorophylls degradation while improved the sugars and acids concentration and preserved pulp carotenoids and total antioxidants during four weeks of storage. However, Put treatment (10^{-3} M) alone did not perform better compared to control fruit. In comparison to CA storage alone, 1-MCP treatment prior CA storage improved pulp carotenoids and sugars to acids ratio during 3 weeks of storage but did not improve firmness or retard softening and its enzyme activities, skin colour rating, skin pigments, total sugars, and total antioxidants within 5 weeks of storage. The combination of 1-MCP treatment and CA storage showed an improvement similar to CA storage alone in overall quality of fruit during ripening following 3 weeks of storage, and prolonged storage to 5 weeks resulted in low quality of ripe fruit.

8.7.Future research

The present study has provided some basic information regarding the maintenance of fruit quality during CA storage in mango. This also opens for further investigation on the following areas:

- The influence of CA storage on the degradation of cell wall matrices and the enzymes activities in softening of 'Kensington Pride' mango needs to be investigated.
- The inhibition mechanism of CA on colour development regarding the chlorophylls and carotenoids degradation and/or synthesis needs further investigation.
- The activation and deactivation of enzymes involve in TCA cycles during storage and ripening of mango fruit as affected by methyl jasmonate treatment in combination with CA storage needs further exploration.
- The concentration of methyl jasmonate and putrescine in 'Kensington Pride' mango during CA storage needs further investigation since their abundance in fruit may influence the ripening process including the colour development, sugars and organic acids biosynthesis.
- The synergistic interaction between 1-MCP and CA storage on fruit metabolism including carbohydrate and bioactive compounds metabolism during storage and ripening are worthy of investigations in the future.

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