

**School of Molecular and Life Sciences**

**Manipulation of Fruit Ripening, Quality and Storage Life in Pome  
Fruits Using Novel Ethylene Antagonists**

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**This thesis is presented for the degree of**

**Doctor of Philosophy**

**of**

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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 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: 26/03/2019 .....

**DEDICATED**

**To**

***My Husband (Asif Ali)***

**For his unconditional love, support and encouragement during the hard times of PhD  
research**

**&**

***My Mother (Mrs. Surraya Yaseen, Late)***

**A virtuous human being, whose prayers helped me to achieve this milestone**

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With Regards,

**Mehwish Yaseen**

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## Abstract

Apple and pear are typical climacteric fruits exhibiting a burst in ethylene production and respiration rate during fruit ripening. Ethylene promotes fruit ripening and senescence as well as shortening the storage life of fruits. The deleterious effects of ethylene lead to huge postharvest losses, hence new strategies to address the future threats of food security are needed. Inhibiting ethylene action is more effective in curtailing postharvest losses than inhibiting ethylene biosynthesis. Therefore, in the present research, the efficacy of some novel ethylene antagonists *viz.*, 1-hexylcyclopropene (1-HCP), (*S*)-(-)-limonene and *trans*-cinnamaldehyde (TCA) was evaluated using 1-methylcyclopropene (1-MCP) as a standard practice. The main objective of this study was to elucidate the effects of these ethylene antagonists on ethylene production, respiration rate, onset of ethylene and respiratory climacteric and fruit quality in apple (cv. 'Fuji' and 'Cripps Pink') and pear (cv. 'Packham's Triumph' and 'Beurre Bosc') under cold and controlled atmosphere (CA) storage conditions. In different experiments, the apple and pear fruits were fumigated with different ethylene antagonists by incubation in hermetically sealed plastic drums. After treatment with ethylene antagonists, apple fruit were stored in cold rooms ( $0.5 \pm 0.5$  °C and  $85 \pm 5$  % R.H.) for 28, 75 and 120 days and in CA comprising of 2 % O<sub>2</sub> and 1 % CO<sub>2</sub> at 1 °C and  $95 \pm 5$  % R.H. for 6 and 8 months. Pear fruit were also stored in cold rooms (0 - 1 °C and  $85 \pm 5$  % R.H.) for 4 and 6 months. At the end of specific storage periods, the fruit were shifted to simulated shelf conditions and rate of ethylene production and respiration rate were determined daily at room temperature ( $21 \pm 1$  °C) until post climacteric phase. SSC, TA, SSC/TA ratio, firmness, fruit peel colour, ascorbic acid, total phenolics, total antioxidants, total and individual sugars and organic acids were also determined on 10<sup>th</sup> day of simulated shelf conditions. After 28, 75 and 120 days cold storage of 'Fuji' and 'Cripps Pink', maximum suppression in climacteric ethylene peak was recorded with 1-MCP ( $126.4 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ) followed by (*S*)-(-)-limonene and TCA ( $142.5$  and  $184.1 \text{ pmol kg}^{-1} \text{ s}^{-1}$  respectively) after 28 days cold storage of 'Fuji' fruit. Ethylene climacteric peaks were delayed for 3.5 - 5.1 days with ethylene antagonists in both apple cultivars. Moreover, 1-MCP reduced the climacteric respiration to a great extent in 'Fuji' and 'Cripps Pink' ( $0.29$  and  $0.26 \text{ } \mu\text{mol kg}^{-1} \text{ s}^{-1}$  respectively). Maximum delayed respiratory climacteric peak was found with (*S*)-(-)-limonene fumigation (2.4 days)

in 'Cripps Pink' apple. TA was significantly highest in 'Fuji' and 'Cripps Pink' fruit (0.38 and 0.84 % respectively) with 1-MCP fumigation while, significantly lowest SSC (12.9 %) was also exhibited by 1-MCP fumigated 'Cripps Pink' fruit. Highest fruit firmness was recorded in 1-MCP treated 'Fuji' and 'Cripps Pink' (76.9 and 79.1 N respectively) followed by TCA treatment in 'Fuji' (72.3 N). Concentrations of ascorbic acid, total phenolics, total antioxidants and individual sugars were also effectively maintained by fumigation treatments as compared to the control. 1-MCP and 1-HCP fumigation delayed the onset of climacteric ethylene peaks by 5 - 6 days in 'Cripps Pink' after 6 and 8 months of CA storage. In 'Fuji' fruit, respiratory climacteric peaks were delayed for 1.7 and 1.3 days with 1-MCP and 1-HCP fumigation respectively after 6 months CA storage as compared to all other treatments and control. Onset of respiratory peaks in 'Cripps Pink' apple was delayed for 1.8, 1.5 and 2.3 days with 1-MCP, (*S*)-(-)-limonene and TCA fumigation respectively. In 'Cripps Pink' and 'Fuji', significantly higher TA was recorded with 1-MCP fumigation treatment (0.60 and 0.34 % respectively). Mean SSC was significantly higher with 1-MCP (14.5 %) and 1-HCP (14.4 %) fumigation treatments in 'Cripps Pink' whereas in 'Fuji' maximum SSC was recorded in 1-MCP treated fruit (12.7 %) followed by (*S*)-(-)-limonene and control fruit (12.5 %). All fumigation treatments significantly reduced the mean SSC/TA ratio in 'Cripps Pink' and 'Fuji' apple as compared to the control. 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA maintained sugar levels, ascorbic acid, total phenolics and antioxidant levels in both apple cultivars. Conversely, organic acids were not significantly affected by ethylene antagonists. The climacteric ethylene production in 'Packham's Triumph' and 'Beurre Bosc' pear was suppressed with 1-MCP (20.9 and 71.6 pmol kg<sup>-1</sup> s<sup>-1</sup> respectively) followed by (*S*)-(-)-limonene in 'Beurre Bosc' (307.7 pmol kg<sup>-1</sup> s<sup>-1</sup>). Onset of climacteric ethylene peaks was also delayed for 1.83 - 3 days with fumigation treatments in both pear cultivars as compared to the control. Rate of climacteric respiration was highly reduced by 1-MCP fumigation in 'Packham's Triumph' and 'Beurre Bosc' pear (0.33 and 0.30 μmol kg<sup>-1</sup> s<sup>-1</sup> respectively). Meanwhile onset of respiratory peak was delayed by 1-MCP and 1-HCP fumigation treatments (2.83 and 2.33 days respectively) in 'Beurre Bosc' pear. Mean fruit firmness of 'Packham's Triumph' pear was significantly higher with 1-MCP fumigation (64.7 N) followed by TCA (34.1 N) while in 'Beurre Bosc' pear, fruit firmness was significantly higher with 1-MCP (60.6 N) and 1-HCP treatments (50.1

N). Generally, all ethylene antagonists reduced the loss of fruit firmness, levels of ascorbic acid and total antioxidants as compared to the control in both pear cultivars. Mean TA of 'Packham's Triumph' fruit was significantly higher in 1-MCP (0.36 %) and (*S*)-(-)-limonene (0.35 %) treated fruit; whereas significantly higher mean TA was recorded with 1-MCP (0.37 %) and significantly higher mean SSC was found in control 'Beurré Bosc' fruit. Comparatively, higher concentration of sucrose (11.5 g kg<sup>-1</sup>) and glucose (9.5 g kg<sup>-1</sup>) was found in 1-HCP and (*S*)-(-)-limonene fumigated 'Packham's Triumph' fruit. In 'Beurre Bosc' pear, concentration of both individual and total sugars was relatively higher with TCA fumigation as compared to other ethylene antagonists and control. In conclusion, the effect of 1-MCP fumigation treatment was more efficient as compared to all other ethylene antagonists and control for extending storage life and fruit quality of tested apple and pear cultivars. 1-HCP, (*S*)-(-)-limonene and TCA also suppressed and delayed climacteric ethylene and respiration peaks compared to control and maintained the fruit quality depending upon storage type, duration and cultivars of apples and pears. 1-HCP, (*S*)-(-)-limonene and TCA seems to have a great potential for antagonising ethylene action, extending the cold and CA storage life and maintaining the quality of apple and pear fruits.

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**List of Symbols and Abbreviations**

×	multiply/interaction
μ	Micro
±	Plus/minus
\$	Dollar
%	Percentage
<	Less than
–	Hyphen
@	At
α	Alpha
β	Beta
1-ECP	1-Ethylcyclopropene
1-MCP	1-Methylcyclopropene
1-OCP	1-Octylcyclopropene
1-PCP	1-Propylcyclopropene
1-PentCP	1-Pentylcyclopropene
1-DCP	1-Decylcyclopropene
1,2-DMCP	1,2-Dimethylcyclopropene
1,3,3-TMCP	1,3,3-Trimethylcyclopropene
2,5-NBD	2,5-Norbornadiene
3-MCP	3-Methylecyclopropene
3,3-DMCP	3,3-Dimethylcyclopropene
a*	Fruit peel red blush
ANOVA	Analysis of variance
APAL	Apple and Pear Australia Ltd.
Ag <sup>+</sup>	Silver ion
AgNO <sub>3</sub>	Silver nitrate
AOA	Aminoxyacetic acid
AVG	Aminoethoxyvinylglycine
ACS	1-Aminocyclopropane-1-carboxylic acid synthase
ACO	1-Aminocyclopropane-1-carboxylic acid oxidase
ABA	Abscisic acid

ABS	Australian bureau of statistics
ACC	1–Aminocyclopropane–1–carboxylate
APX	Ascorbate peroxidase
b*	Fruit peel yellowness
cm	Centimetre (s)
cv.	Cultivar
CH <sub>3</sub>	Methyl
°C	Degree Celsius
Co <sup>2+</sup>	Cobalt ion
CO <sub>2</sub>	Carbon dioxide
CP	Cyclopropenes
C*	Chroma
CA	Controlled atmosphere
CS	Cold storage
CAT	Catalase
CRD	Completely randomized design
CPAS	3-Cyclopropy, 1-1-enyl-propanoic acid sodium salt
Cu	Copper
d	Days
d.H <sub>2</sub> O	Distilled water
DACP	Diazocyclopentadiene
DPA	Diphenylamine
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DAFWA	Department of Agriculture and Food Western Australia
e.g.	Latin <i>exempli gratia</i> , meaning for example
et al.	Latin word; <i>et alia</i> meaning ‘and others’
EDTA	Ethylenediaminetetraacetic acid
EPA	Environment Protection Agency
ER	Endoplasmic reticulum
FAO	Food and agriculture organisation
FFC	Fruit flavour complex
g	Gram
GRAS	Generally Recognized As Safe

h	Hour
h°	Hue angle
1-HCP	1-Hexylcyclopropene
HAL	Horticulture Australia Ltd.
HPLC	High pressure liquid chromatography
K <sub>i</sub>	Inhibition constant
Kg <sup>-1</sup>	Per kilogram
KI	Potassium iodide
L*	Lightness
L	Litter
L <sup>-1</sup>	Per litre
Ltd.	Limited
LSD	Least significant difference
mg	Milligram (s)
m <sup>2</sup>	Per meter square
ml	Milliliter (s)
min	Minute (s)
Milli-Q	Trademark for ultrapure water
M	Month
MAP	Mitogen Activated Protein
MVG	Methoxyvinylglycine
MW	Molecular weight
MT	Million tonne (s)
MTA	Methylthioadenine
nmol	Nanomolar (s)
nL	Nanolitre (s)
N	Newton
NS	Non-significant
NaF	Sodium fluoride
NaOH	Sodium hydroxide
NSW	New South Wales
<i>P</i>	Probability level
pmol	Picomolar (s)

PG	Polygalacturonase
PME	Pectin methyl esterase
PEL	Pectate lyase
ppm	Parts-per-million
ppb	Parts-per-billion
PPO	Polyphenol oxidase
RA	Regular air
RI	Refractive index
rpm	Revolutions per minute
RH	Relative humidity
RNA	Ribonucleic acid
ROS	Reactive oxygen species
s <sup>-1</sup>	Per second
SAM	S'adenosyl-l-methionine
SOD	Superoxide dismutase
SP	Storage period
SE	Standard error
SSC	Soluble solids concentration
STS	Silver thiosulphate
T	Treatment
TCA	<i>trans</i> -Cinnamaldehyde
TCO	<i>trans</i> -Cyclooctene
T	Tonnes
TA	Titrateable acidity
TA/SSC	Titrateable acidity/ soluble solids concentration ratio
μmol	Micromolar
UK	United Kingdom
USA	United States of America
UV	Ultra-violet
v/v	volume/volume
WA	Western Australia
WAPA	The World Apple and Pear Association
WTE <sub>x</sub>	World Top Exports

WS-CPD      Water soluble CP derivative

## CHAPTER 1

### General Introduction

Apple (*Malus × domestica* Borkh.) and pear (*Pyrus communis* L.) together constitute a highly valued fruit industry of Australia after citrus, banana and grapes, and is worth 681 million dollars (Apple and Pear Australia Limited [APAL], 2018). Globally, China is the leading producer of apples and pears both in terms of production volume and value (FAOSTAT, 2016). In Australia, total production of apple and pear is 295,196 and 105,243 tonnes respectively with major production from Victoria (45 % and 89 % respectively); while Western Australia contributes approximately 9 % apple and 1 % pear to the total Australian production. Major production areas of Western Australia lie in the Perth Hills, Donnybrook and Manjimup regions with a wide range of cultivars which are available in markets around the year (APAL, 2018). Australian share in global markets is very minor and total export volume of apples is also very low constituting 1-2 % of total production to overseas markets of United Kingdom, Asia, United Arab Emirates and Thailand. However, 16.5 % of pear production is exported to New Zealand, Indonesia, Hong Kong and Singapore (AgriFutures Australia, 2017).

Perishability of horticultural commodities is a great challenge for their profitable marketing in terms of packaging, storage and transportation (Ansari and Tuteja, 2015). Inadequate handling and control of storage environment can lead to postharvest losses which refers to the losses in fruit quality and quantity during the supply chain (Buzby *et al.*, 2014). Fresh horticultural produce losses vary from 40-50 % along the supply chain from production to the retail phase of marketing in different parts of the world (Gustavsson *et al.*, 2013; FAO, 2015; Flores-Lopez *et al.*, 2016). Moreover, Lipinski *et al.* (2013) reports that one out of four calories produced are never consumed by end users due to postharvest losses. These losses lead to considerable wastage of resources and ultimately pose a threat to profitability of all stakeholders (Gogo *et al.*, 2017). Recent advances in postharvest handling of the supply chain facilitate to meet the global criteria of better quality horticultural produce with high nutritional value by using a number of methods including both chemical and physical (Mahajan *et al.*, 2014). However, an increasing world population, and its progressively scarce resources, offer an attractive opportunity to revitalise the

postharvest technologies for a sustainable food supply around the globe in future (Rosegrant *et al.*, 2015).

Ethylene provokes a variety of plant growth and developmental processes including fruit ripening (Song *et al.*, 2018). Both endogenous and exogenous ethylene influence the physiology and biochemistry of fruits through the expression of specific genes involved in ripening and senescence (Yang *et al.*, 2013; Mattoo and Suttle, 2017). In climacteric fruits, all the primary features of ripening including volatile production, softening, and pigment changes, are inexorably linked to ethylene perception and signal transduction (Munoz-Robredo *et al.*, 2012; Cherian *et al.*, 2014). Presence of ethylene in the supply chain hastens ripening of climacteric fruits and leads to fruit decay and waste (Blanke, 2014; Song *et al.*, 2018). Once harvested, fruit quality declines rapidly due to autocatalytic ethylene biosynthesis, leading to reduced shelf life, microbial degradation and a variety of physiological disorders. Ethylene promotes a series of ripening and senescence events by binding reversibly to specific receptor sites in the plants which contain copper as a transition metal cofactor (Wills and Golding, 2016). Adverse effects of ethylene on fresh fruits and vegetables vary and include senescence, chlorophyll loss, softening, sprouting, abscission of leaves and flowers, discoloration and toughening of vegetables (Goren *et al.*, 2011; Wills and Golding, 2016). Apple and pear are climacteric fruits exhibiting a sharp rise in endogenous ethylene production and respiration during ripening leading to changes in texture, flavour, aroma and reduced shelf life at ambient conditions, which necessitates modification in storage atmosphere, reduced exposure to ethylene during storage/transportation and inhibition of ethylene action (Pech *et al.*, 2012; Paul *et al.*, 2012; Tucker *et al.*, 2017).

Postharvest storage techniques, including both cold and controlled atmosphere (CA) storage which are commonly practised for long term storage of pome fruits, sometimes fail to meet the expectations of consumers because of various quality issues. For example, storage life in pear is minimized by internal browning, skin yellowing and scald, similarly, loss of firmness, off-flavour, internal browning and water core are common in apple cultivars during storage. Low oxygen and high carbon dioxide levels in CA storage are also associated with many injuries which adversely affect fruit quality (Thewes *et al.*, 2017). Previous studies reveal that there is a possibility to overcome these issues with the exogenous application of ethylene

antagonists in combination with a precise control of storage atmosphere (Fawbush *et al.*, 2008; Singh *et al.*, 2009; Watkins and Nock, 2012; Valero *et al.*, 2013; Argenta *et al.*, 2016).

There are certain chemicals which inhibit ethylene perception and hold the promise of retarding ripening, senescence, and other adverse effects of ethylene in fresh horticultural produce. Some of these are antagonists which block the ethylene receptors by competing with ethylene and ultimately the signal transduction pathways either reversibly or irreversibly (Jung and Watkins, 2011). An extensive range of compounds and their derivatives, counteracting ethylene at the receptor level have been discovered and assessed including silver ion ( $\text{Ag}^+$ ), silver thiosulfate (STS), 2,5-norbornadiene (2,5-NBD), diazocyclopentadiene (DACP), *trans*-cyclooctene (TCO) and cyclopropenes (CPs) (Sisler, 2006; Seglie *et al.*, 2011). Many previous studies documented that these compounds counteract ethylene responses at molecular level in fresh horticultural produce such as delayed softening, reduced chilling injury, delayed development of volatiles and alcohols, retarded decline in proteins in senescing fruits and prevention of an increase in electrolytic leakage, which ultimately extends storage life (Goren *et al.*, 2011). 1-Methylcyclopropene (1-MCP) has been used extensively to delay the physiological and metabolic effects of ethylene in horticultural commodities due to its high reactivity and commercial availability as a stable formulation. Different studies show that ethylene production, respiration rate and ripening were delayed in various horticultural crops with the application of 1-MCP (Watkins, 2008; Huber, 2008; Lu *et al.*, 2013a; Han *et al.*, 2015; Argenta *et al.*, 2016). However, there are some drawbacks of 1-MCP and other ethylene antagonists which limit their effective use such as the efficacy of 1-MCP varies greatly with temperature, storage conditions, maturity stage of fresh produce and genotype as well as its very expensive (Villalobos-Acuna *et al.*, 2010; Tucker *et al.*, 2017). Moreover, 1-MCP application has been associated with lack of aroma in apples and increased susceptibility to flesh browning after prolonged storage (Watkins and Nock, 2004; Lu *et al.*, 2013a). Certain limitations are also posed by previously used ethylene antagonists including toxicity of heavy metal such as silver ions in edible crops (Apelbaum *et al.*, 2008), obnoxious odour and corrosive nature of 2,5-NBD and explosiveness of DACP at elevated levels. In addition, most of these ethylene antagonists require continuous exposure with higher concentrations and diffuse rapidly counteracting the ethylene effects for a very short

time. Hence, these compounds cannot be used at a commercial scale for long term protection of horticultural commodities (Sisler *et al.*, 2009).

CPs are the versatile organic compounds which have been shown by earlier studies as effective blocking agents for ethylene receptors (Sisler *et al.*, 2010). Recently, a range of CP derivatives have been developed, which are competent in preventing the lethal effects of ethylene similar to 1-MCP (Xu *et al.*, 2016). 1-Hexylcyclopropene (1-HCP), a CP derivative, has been used in scientific and practical attempts advocating that it can effectively manipulate ethylene action involving fruit ripening processes (Serek *et al.*, 2007; Khan *et al.*, 2016) and flower abscission in waxflower (Abdalghani, 2017). Moreover, essential oils from plants have also been used in postharvest studies due to their antimicrobial activities for enhancing shelf life of produce such as cinnamon oil, which contains ‘*trans*-cinnamaldehyde’ as a major constituent and ‘limonene’, a monoterpene, from aromatic plants such as lemon (*Citrus limon* Osbeck) and aztec sweet herb (*Lippia scaberrima* L.) (Regnier *et al.*, 2008; Xing *et al.*, 2011). However, these phytochemicals have not been tested as ethylene antagonists in spite of their positive influence on keeping postharvest quality of fresh produce (Regnier *et al.*, 2010; Lu *et al.*, 2013b; Zhao *et al.*, 2015; Carvalho *et al.*, 2016). Screening of potential ethylene antagonists is warranted to provide cheaper, easily accessible, and more universally applicable compounds to the pome fruit industry as a cheaper alternative to 1-MCP. Moreover, evaluation of cultivar specific responses towards new ethylene antagonists will help their exploitation by commercial growers to extend storage life and maintain quality of pome fruits. It will ultimately lead to better quality fruit supply to consumers and improve profits to all stakeholders of the horticulture industry.

Hence, this research project aimed to evaluate the potential of some new compounds as ‘ethylene antagonists’ in comparison to 1-MCP which could be used to extend postharvest life and maintain quality of pome fruits. For this purpose, efficacy of these compounds was assessed in regulating ethylene production, respiration rate, fruit ripening, softening, extending the cold and CA storage life and quality maintenance of apple and pear fruits. Specific objectives of this research study are as follows;

- To test the efficacy of some innovative ethylene antagonists on ethylene production, respiration rate, extending cold storage life and maintaining quality of 'Fuji' and 'Cripps Pink' apple fruit.
- To assess the efficiency of new ethylene antagonists on modulation of ethylene production, respiration rate, CA storage life and quality of 'Fuji' and 'Cripps Pink' apple fruit.
- To investigate the impact of non-conventional ethylene antagonists on regulation of ethylene production, respiration rate, cold storage life and quality of 'Packham's Triumph' and 'Beurre Bosc' pear fruit.

## CHAPTER 2

### General Review of Literature

#### 2.1. Introduction

Among pome fruits, apple and pear are widely cultivated around the globe due to their commercial importance and are considered as a functional food with reference to their nutritional profile (Kevers *et al.*, 2011; Reiland and Slavin, 2015). With an increasing health consciousness, consumers are more concerned with the content of phytochemicals fruits/vegetables which have specific impact on human health besides nutrition (Kou *et al.*, 2014). Apples are a rich source of dietary fibre and contain a considerable number of bioactive compounds like polyphenols, carotenoids and flavonoids with higher antioxidant activity, equivalent to 1500 milligrams of vitamin C and contributes to preventing various types of cancers (Sudha *et al.*, 2007). A range of trials using apples and apple components have proved that asthma, cardiovascular and gastrointestinal diseases can also be controlled by this fruit (Hyson, 2011). Moreover, apples have been reported to contain triterpenoids such as oleanolic acid and ursolic acid which are anti-inflammatory and hepatoprotective (Lv *et al.*, 2013). Similarly, pears are also a rich source of beneficent compounds including phenolics, amino acids, flavonoids and antioxidants (Kou *et al.*, 2014). Consumption of fresh and processed pears is practised worldwide, and they are particularly used for their anti-hyperglycaemic, laxative and diuretic activities (Reiland and Slavin, 2015).

Globally, China is the chief producer of apples, pears and table grapes and its production has exhibited an upward trend continuously since 2002 (FAOSTAT, 2016). The top ten producers, exporters and importers of apple and pear around the globe are listed in Tables 2.1-2.5. Total production of apples in Australia was approximately 308,299 tonnes for the year 2015-16; meanwhile WA produced about 34377 tonnes during this year. In the case of pears, total Australian production including nashi was 104928 tonnes and the contribution of WA was 4425 tonnes [Australian Bureau of Statistics (ABS), 2017]. Production of apple and pear in Australia varies in different states as presented in Table 2.6. Moreover, Australian exports of apple and pear during 2017 are shown in Figure 2.1.

Table 2.1. Top 10 world apple producers with estimated production values during 2013.

Ranking	Country	Production (Tonnes)
1.	China	31,747,294
2.	United States of America	3,265,286
3.	Turkey	2,502,760
4.	Poland	2,468,059
5.	Italy	1,773,570
6.	India	1,532,000
7.	France	1,389,986
8.	Chile	1,367,671
9.	Iran	1,354,696
10.	Russia	1,257,600

(WAPA Association, 2017)

Table 2.2. Top 10 world pear producers with estimated production values during 2013.

Ranking	Country	Production (Tonnes)
1.	China	15,696,676
2.	United States of America	716,001
3.	Italy	668,726
4.	Argentina	650,092
5.	Turkey	415,643
6.	Spain	383,130
7.	South Africa	308,883
8.	India	306,000
9.	Netherlands	294,300
10.	Belgium	274,500

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(WAPA Association, 2017)

## Chapter 2: General Review of Literature

Table 2.3. Major apple exporters of the world with estimated export values during 2016.

Ranking	Country	Export Value (US\$)	Share in total world export (%)
1.	China	1.5 billion	20
2.	United States of America	936.4 million	13
3.	Italy	917.2 million	12.8
4.	Chile	663.6 million	9.2
5.	France	591.3 million	8.2
6.	New Zealand	494.7 million	6.9
7.	South Africa	358.7 million	5
8.	Poland	313.1 million	4.4
9.	Netherlands	208.3 million	2.9
10.	Belgium	134.1 million	1.9
11.	Serbia	126.8 million	1.8
12.	Japan	122.5 million	1.7
13.	Spain	113 million	1.6
14.	Argentina	73.7 million	1
15.	Austria	63.6 million	0.9

[World Top Exports (WTE<sub>ex</sub>), 2017]

Table 2.4. Major pear exporters with estimated export volumes during 2010

Ranking	Country	Export Volume (MT)
1.	China	437 929
2.	Argentina	419 587
3.	Netherlands	349 324
4.	Belgium	295 406
5.	South Africa	186 616
6.	United States of America	159 291
7.	Italy	134 037
8.	Spain	129689
9.	Chile	116 765
10.	Portagal	88 389

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(Retrieved from; [www.novagrim.com](http://www.novagrim.com))

Table 2.5. Major apple and pear importers of the world with estimated volumes during 2014.

Apple		Pear	
Country	Import volume (T)	Country	Import volume (T)
Germany	621,771	Brazil	208,346
United Kingdom	445,937	Germany	170,699
Belarus	397,588	United Kingdom	167,331
Netherlands	356,195	Netherlands	161,877
Spain	247,088	France	125,394
Mexico	235,502	Belarus	112,500
Canada	222,058	Italy	104,659
United States	207,994	Lithuania	101,913
India	204,570	Mexico	86,969
France	153,702	United States	81,863

(UN COMTRADE Statistics)

Table 2.6. Production of apple and pear in different states of Australia during the year 2014-15.

Australian State	Apple Production (%)	Pear Production (%)
Victoria	45	89
New South Wales	16	<1
Queensland	10	<1
Tasmania	9	4
South Australia	10	5
Western Australia	9	1

(Source: ABS, 2017 retrieved from; apal.org.au)

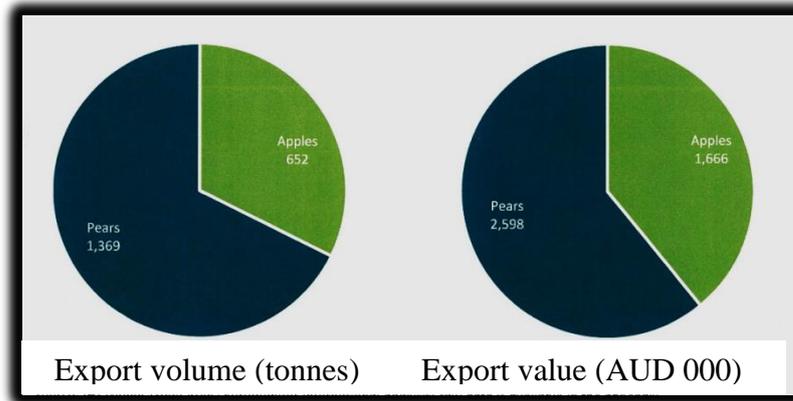


Figure 2.1. Statistics for Australian apple and pear export during Quarter 1 of 2017 (Hort Innovation, 2017).

## 2.2. Economic Cultivars of Apple and Pear

A wide range of apple and pear cultivars are grown in Australia which are marketed round the year and have great diversity in terms of colour, shape, size and taste (APAL, 2018) as shown in Figure 2.2. Varieties with red colour have most market value due to additional anthocyanin content, an antioxidant which has a beneficial effect on human health (Crawford, 2017). Apples are harvested in Australia from February till May and major commercial varieties include Cripps Pink (Pink Lady<sup>®</sup>), Cripps Red (Sundowner<sup>™</sup>), Granny Smith, Royal Gala, Fuji, Golden Delicious and Red Delicious. Cripps Pink and Cripps Red were developed and released in Australia (Department of Agriculture and Food, Western Australia [DAFWA], 2006). Some newly released apple cultivars are Jazz, Envy, Bravo, Modi and Eve (APAL, 2018). In Australia, both European and Asian pears are grown, however, most commercial varieties are European pears. One of the highly cultivated pears is ‘William’s Bon Chrétien’ while other economic cultivars include Beurré Bosc, Red Anjou, Josephine de Malines, Packham’s Triumph, Red Sensation and Winter Nelis (AgriFutures Australia, 2017).

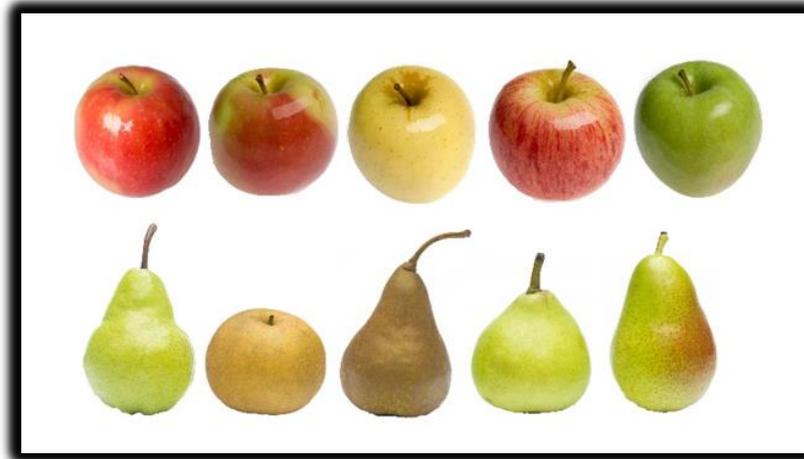


Figure 2.2. Diversity of Australian apple and pear cultivars in terms of size, shape and colour.

### 2.3. Postharvest Food Losses

Commercial horticulture is one of the most profitable disciplines of agriculture; however, postharvest losses vary from 15-50 % because of the highly perishable nature of horticultural commodities (Gustavsson *et al.*, 2013; Romanazzi *et al.*, 2017). According to an estimate, one third of the fruits and vegetables produced never reach consumers due to these losses (Kader, 2005). Fresh horticultural commodities face losses through the entire supply chain right from the production farms to final consumers. It was reported by the Food and Agriculture Organisation that global food losses account for 1.3 billion tonnes on a yearly basis (Gustavsson *et al.*, 2013). Percentage of postharvest losses in various commodities have also been reported by Lipinski *et al.* (2013) and presented in Figure 2.3. Food losses refer to a decline in quality or quantity and is contributing to an increasing hunger worldwide. Moreover, food losses have a severe negative impact on the environment as production of food utilizes excessive resources like land, water, energy etc. (FAO, 2015). As the processes involved in postharvest degradation of produce are not yet well understood, progress in reducing postharvest losses and extending the shelf life of fresh produce has been limited (Nambeesan *et al.*, 2010). In commercial horticulture, huge annual losses urge to study the molecular and physiological aspects responsible for the susceptibility of plants to biotic and abiotic stresses, poor fruit quality and reduced shelf life of economically important crops. To feed the ever-increasing world population, the postharvest technology procedures need to be optimized to minimize

the spoilage and degradation of fresh produce (Kader, 2008). Hence, postharvest technology offers strategic ways to provide food security, and to raise the income of agricultural stake holders.

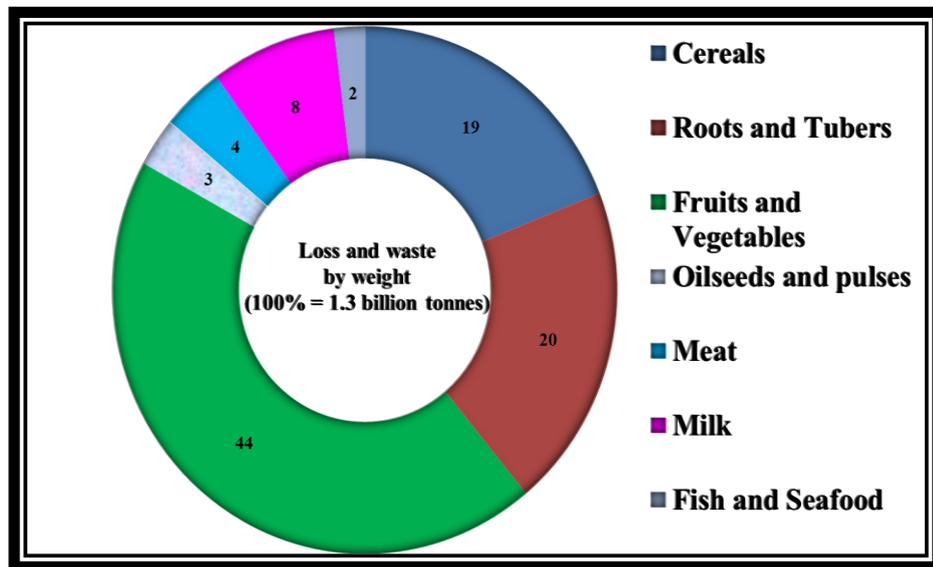


Figure 2.3. Percentage of global food losses and waste by commodity  
(Lipinski *et al.*, 2013)

### 2.4. Physiological Mechanism of Fruit Ripening and Role of Ethylene

Fruits are an important part of human diet as they provide all the essential nutrients of a balanced diet and postharvest life of fruits is one of the important criteria determining their shelf life. Ripening adds value by influencing the level of pigments, sugars, acids, and aroma, making product more attractive and palatable (Matas *et al.*, 2009). Increased softness and susceptibility to microbial infection in ripe produce cause reduction in overall shelf life. Several internal and external factors stimulate fruit ripening including physical environment, nutrient availability, water status and plant growth regulators (Giovannoni, 2007). Ethylene is a well-known phyto-hormone which regulates a variety of developmental processes in plant's life cycle and contributes to a plant's responses to biotic as well as abiotic stresses (Iqbal *et al.*, 2013). It is a simple gaseous compound which has been extensively studied for its biosynthetic, metabolic and signalling pathways in plants (Yang and Hoffman, 1984; Kende, 1993; Wang *et al.*, 2002; Guo and Ecker, 2004; Barry and Giovannoni, 2007; Yoo *et al.*, 2009; Iqbal *et al.*, 2013). It is one of the five basic plant hormones produced by all the plant species and expressed by 7 % of the genome (Czarny *et al.*, 2006).

Autocatalytic synthesis of ethylene has been shown to influence the physiology and biochemistry of fruits by the expression of specific genes involved in ripening (Halton, 2009). The essential role of ethylene in the regulation of fruit ripening and the relevant processes has been revealed by earlier studies (Klee *et al.*, 1991; Picton *et al.*, 1993; Bleecker and Kende, 2000; Giovannoni, 2001; Barry and Giovannoni, 2007). Maximum postharvest losses of perishable commodities are referred to ethylene induced ripening and both endogenous as well as exogenous ethylene has potential to accelerate these physiological processes in plants (Feng *et al.*, 2004). Production of this ripening gas within plants changes according to developmental stages and physiological status (Yang and Hoffman, 1984). There are various exogenous sources of ethylene such as engine exhausts, pollutants and other waste materials, rotten plant products and microorganism; which may cause damaging effects when plants are exposed to them at any stage of the supply chain (Cape, 2003). However, sensitivity towards ethylene varies greatly among plant species and depends upon several factors including ethylene concentration and exposure time (Wills *et al.*, 2001). Common physiological damaging effects include enhanced fruit metabolism, loss of weight, juice leakage and flesh browning. Ethylene causes an increase in the gene expression and activity of cell wall targeted enzymes e.g. polygalacturonase (PG) resulting in fruit softening (Trainotti *et al.*, 2003; Hayama *et al.*, 2006). Based on ethylene biosynthesis and respiration rates, fruits are classified as climacteric and non-climacteric. Climacteric fruits are characterized as having an exponential increase in ethylene production and a burst in respiration rate which correlates with the development of fruit flavour complex (FFC). Moreover, exogenous ethylene application stimulates ripening related attributes in climacteric fruits but not in non-climacteric. Difference between climacteric and non-climacteric ripening is exhibited in Figure 2.4.

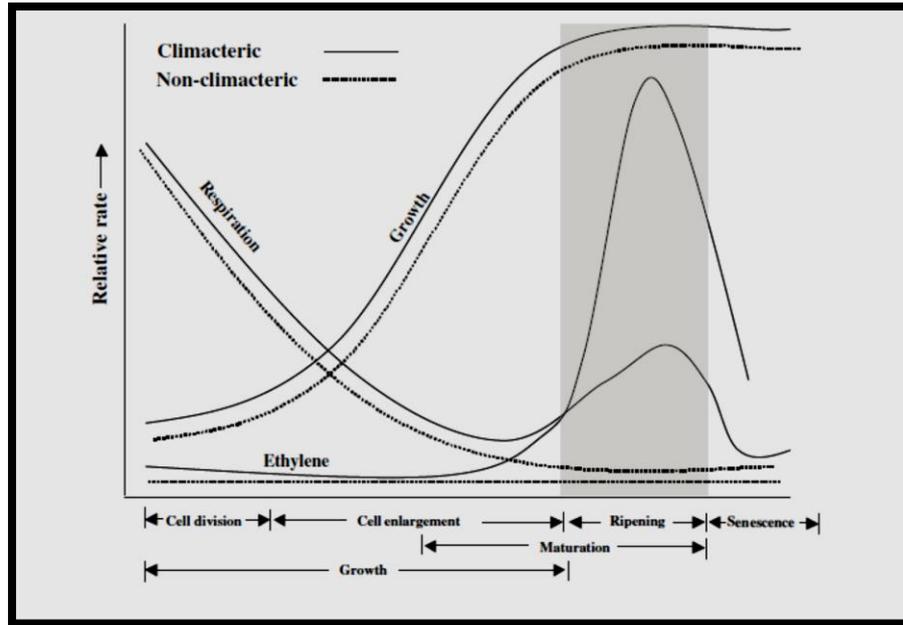


Figure 2.4. A burst in ethylene production and respiration rate during climacteric ripening distinguished from non-climacteric ripening (Paul *et al.*, 2012).

There are very few studies which support the role of ethylene in development and ripening of non-climacteric fruits (Rhodes, 1980; Huber, 2008) like de-greening of citrus (Abeles *et al.*, 1992) and anthocyanin synthesis in grape berries (Weaver and Montgomery, 1974; Tonutti *et al.*, 2007). Besides a positive role in fruit quality, ethylene regulated ripening imparts a lot of negative attributes by making the fresh produce susceptible to microbial infection and reducing the shelf life. Climacteric fruits such as apple, pear, peach, banana and kiwifruit produce ethylene at higher rates and show increased sensitivity to exogenous ethylene as well. Some non-climacteric fruits like strawberry and persimmon are also highly sensitive to ethylene (Wills and Warton, 2000). Responses of plants and their produce are highly variable to endogenously produced and exogenously supplied ethylene. Detrimental effects of ethylene spoiling fruit quality include various physiological disorders (such as chilling injury, superficial scald and internal browning), off-flavours such as in banana, discoloration (e.g. avocado) and softening in apple, mango, avocado, kiwifruit, strawberry and melon (Karakurt and Huber, 2002; Johnston *et al.*, 2002; Pesis *et al.*, 2002; Salvador *et al.*, 2004; Bower *et al.*, 2003; DeLong *et al.*, 2004; Jeong and Huber, 2004).

## 2.5. Postharvest Approaches to Regulate Fruit Ripening and Quality

Manipulation of the ripening process is a great challenge for producers aiming to supply quality fruits to the consumers with desirable colour, firmness, taste and texture. However, tremendous progress has been made in postharvest technology to provide superior quality produce such as better packaging, advanced transport and storage systems, disease and pest control, manipulation of ripening process and optimization of the supply chains (Scariot *et al.*, 2014). As ethylene is a key player regulating the ripening and senescence in plants, shelf life can be greatly enhanced by manipulating the ethylene biosynthesis within plants using novel postharvest technologies and huge losses of horticultural produce can be prevented (Grichko, 2006; Matas *et al.*, 2009). Mechanism of ethylene biosynthesis in plants is exhibited in Figure 2.5. In addition, ethylene can be used beneficially in the horticulture sector in an extensive way by regulating its biosynthesis and action (Opiyo and Ying, 2005). The minimally processed fruit industry dealing with the marketing of fresh cut slices is also in great need of ethylene regulation as peel removal leads to quick decline in quality because of degradative processes, microbial growth and browning (Perera *et al.*, 2003).

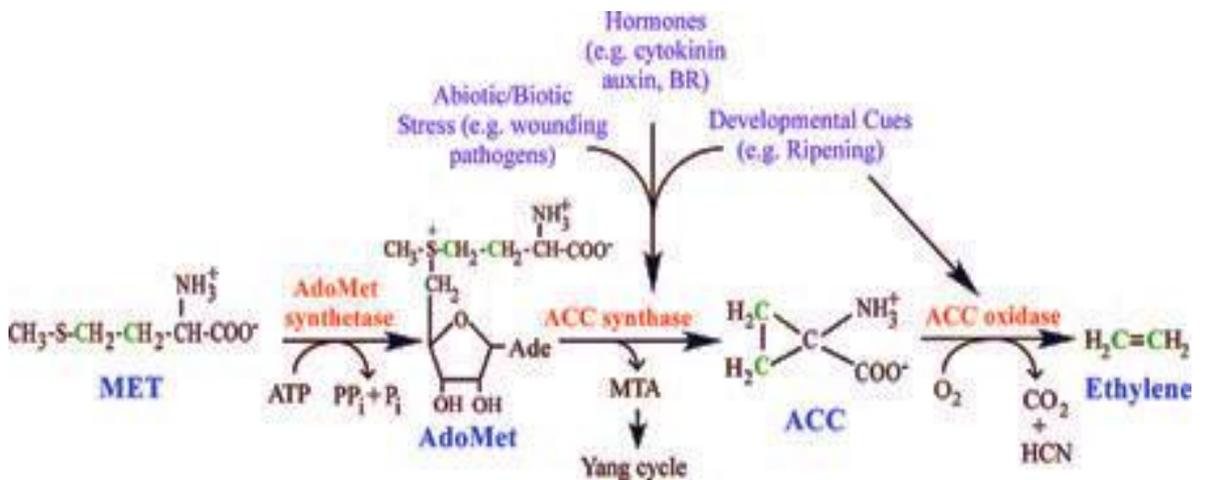


Figure 2.5. Biosynthesis of Ethylene. AdoMet: S-adenosyl-methionine; Met: methionine; ACC: 1-aminocyclopropane-1-carboxylic acid; MTA: methylthioadenine (Argueso *et al.*, 2007).

## 2.6. Regulation of Ethylene Action

There are basically two approaches to control adverse effects of ethylene. Firstly, it can be achieved by limiting ethylene biosynthesis by suppressing activities of key enzyme such as ACS (1-aminocyclopropane-1-carboxylate synthase) and ACO (1-aminocyclopropane 1-carboxylate oxidase) using aminoethoxyvinylglycine (AVG), methoxyvinylglycine (MVG), aminoxyacetic acid (AOA),  $\text{Co}^{2+}$ , ethanol,  $\alpha$  aminoisobutyric acid. This approach is not very beneficial as it does not protect plants from exogenous sources of ethylene (Lau and Yang, 1976; Baker *et al.*, 1978; Reid and Wu; 1992; Martinez-Romero *et al.*, 2007). Secondly, in recent years, the most effective is the use of inhibitors to control ethylene action which protects fresh produce from endogenous and exogenous sources of ethylene (Serek and Reid, 1993; Sisler *et al.*, 2006). Ethylene action starts with its binding to the ethylene receptors containing copper. The receptor sites send signals through a pathway including a MAP (Mitogen Activated Protein) kinase cascade, a metal transporter intermediate, and a transcriptional cascade which ultimately stimulate a specific biological action (Bleecker and Kende, 2000). Five genes *viz.*, ETR1, ETR2, EIN4, ERS1 and ERS2 are responsible for encoding ethylene receptor (Figure 2.6) (Bleecker and Kende, 2000; Yoo *et al.*, 2009). Different compounds bind to the receptor in a different way including agonists and antagonists which can be differentiated using a biological assessment (Sisler, 2006). A model for the functioning of the ethylene receptor and its binding has been presented by Pirrung (1999). Ethylene inhibitors or antagonists control/inhibit ethylene action by blocking the receptor sites and ultimate signal transduction pathways (Sisler and Serek, 1997; Goren *et al.*, 2008). Hence, shelf life and quality attributes of fruits can be greatly enhanced using effective ethylene inhibitors. This approach to minimize food losses can be more economical and environmentally friendly than increasing crop production using marginal lands and other diminishing resources (Gogo *et al.*, 2017).

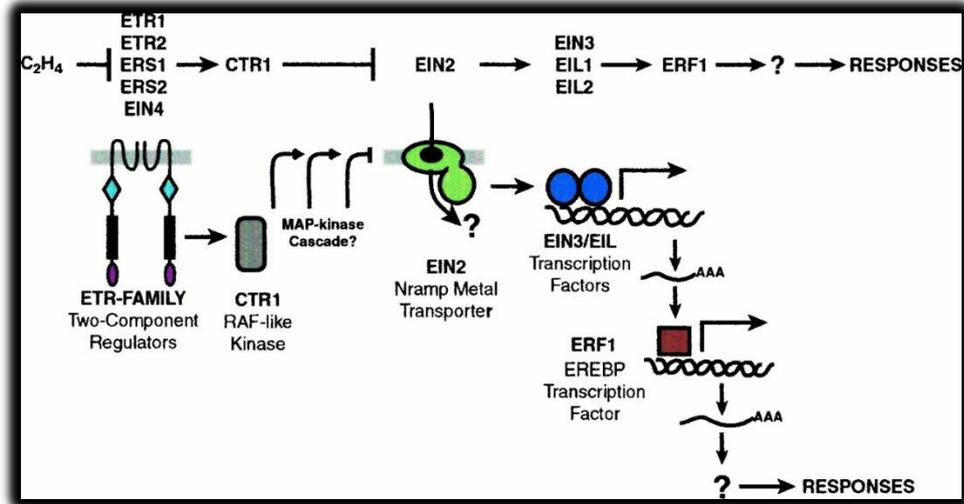


Figure 2.6. Ethylene signal transduction pathway

**Interpretation from left to right**, ethylene regulates negatively a family of membrane-associated receptors i.e. ETR1, ETR2, ERS1, ERS2, EIN4. The histidine-kinase transmitter domains of receptor family interact with the Raf-like kinase CTR1. This receptor (CTR1 complex) negatively regulates a membrane protein (EIN2) related to a superfamily of metal transporters. The cytoplasmic C-terminal domain of EIN2 positively signals downstream to the EIN3 family of transcription factors located in the nucleus. A target of the EIN3 transcriptions factors is the promoter of the ERF1 gene, a member of a second family of transcription factors. ERF1 is rapidly induced in response to ethylene and can activate a subset of ethylene responses when ectopically expressed (Bleecker and Kende, 2000).

## 2.7. Ethylene Antagonists

There is evidence that a metal containing receptor is responsible for ethylene action and many compounds have an ability to bind with this receptor including agonists and antagonists (Pirrung *et al.*, 2008). Sisler *et al.* (2006) have classified the compounds binding to ethylene receptors in 3 categories: 1) Agonists, compounds which bind to receptors and generate responses similar to ethylene e.g. propene, carbon monoxide, acetylene; 2) Antagonist, compounds which compete for binding with ethylene and inhibit ethylene responses in plants but require continuous exposure to be effective e.g. cyclic olefins including 2,5 NBD; 3) Irreversible antagonist compounds, which bind with receptors for a very long time only by a single exposure

and have more affinity for binding sites than ethylene e.g. cyclopropenes. Ethylene antagonists principally block the receptor sites to control the ethylene action and diffuse after some time (Blankenship and Dole, 2003). Sisler *et al.* (2006) reported that these compounds lock the receptor in an inactive state. Some antagonistic compounds interact with the receptors reversibly while some interact irreversibly (Halton, 2009). Difference in the diffusion time of different antagonists differentiates among them and is considered as a major factor to determine the potency of ethylene inhibitors. The ethylene receptor is omnipresent in plants, hence; these compounds are supposed to counteract ethylene action in all plant species (Burg and Burg, 1967; Sisler and Serek, 1997; Sisler and Serek, 1999). Sisler and Serek (1997) have reviewed various aspects of these compounds. They can greatly reduce the spoilage of fruits, herbs, leafy vegetables and ornamentals at all stages of the supply chain. Ripening related genes are suppressed using ethylene inhibitors which leads to extended storage (Martinez-Romero *et al.*, 2003). Sisler and his colleagues have made a great contribution as they have discovered and assessed a wide range of compounds and their derivatives counteracting ethylene at the receptor level (Sisler and Serek, 1999). The number of compounds is increasing day by day in this context for scientific research as well as for practical applications (Sisler *et al.*, 2006). Discovery of ethylene antagonists has led to new advances in plant biology by giving a detailed understanding of ethylene receptors, signal transduction pathways, mechanism of gene action as well as discovery of some new genes.

Most commonly used inhibitors include  $\text{Ag}^+$  ion (Beyer, 1976), STS (Veen and van der Geijn, 1978), cyclic olefins, 2,5-NBD (Sisler *et al.*, 1985), DACP (Sisler and Blankenship, 1993; Serek *et al.*, 1994), diphenylamine (DPA) (Wang and Dilley, 2000) *trans*-cyclooctene (TCO), cyclopropene (CP), 3,3-dimethylcyclopropene (DMCP) (Sisler, 2006) and 1-MCP (Serek and Sisler, 2005).  $\text{Ag}^+$  ion was the first inhibitor of ethylene perception reported and has been used very effectively in ornamental crops (Huber, 2008). Kofranek and Paul (1975) used it in their experiments in the form of silver nitrate ( $\text{AgNO}_3$ ). Moreover, it has been used extensively for many years in the form of STS because of its easy uptake and high mobility within plants (Veen and van de Geijen, 1978; Veen, 1983). STS and DACP were used for the first time to demonstrate the suppression of ethylene perception and interruption of senescence in both fruit and floral organs (Tucker and Brady, 1987). STS was used in

fruit and vegetable crops (Atta-Aly *et al.*, 1987; Perkins-Veazie *et al.*, 1996) but, later the application of silver compounds was blocked in edible crops because of heavy metal toxicity (Reid and Staby, 2008).

Potential of alkenes to inhibit ethylene induced detrimental effects was discovered by Sisler and Pian (1973). Cyclic alkenes with higher proficiency level as an ethylene inhibitor are; DACP, TCO, *cis*-cyclooctene, 2,5-NBD, cyclopentadiene, 4-pentene-1-ol, cyclopentene, cyclohexene, allylbenzene, norbornene, 1,3-cyclohexadiene, 1,4-cyclohexadiene, 1,3-cycloheptadiene, 2-allylphenol and 4-phenyl-1-butene (Sisler *et al.*, 2006). However, the most extensively used compounds in scientific studies are, 2,5-NBD, DACP, TCO and 1-MCP (Sisler and Serek, 1999). 2,5-NBD has been used extensively in research studies and proved to be a potent inhibitor. However, it has a limitation of having pungent and abhorrent smell making its use difficult (Reid and Staby, 2008). Secondly, it needs continuous exposure to be effective and stimulates the production of ethylene at higher concentrations (Sisler *et al.*, 2003). It is commercially available and has been tested on different horticultural plants previously (Sisler *et al.*, 1985; Serek *et al.*, 2007). Cyclopentadiene also has an antagonistic effect like 2,5-NBD. (Sisler and Serek, 1999; Sisler *et al.*, 2006). TCO also competes with ethylene for binding to the receptor sites and binds for a longer time (about 3-6 hours) compared to ethylene. They are also a better choice with respect to effective concentration. Larsen and Chang (2001) and Hirayama *et al.* (1999) have found antagonistic activity of both NBD and TCO in arabidopsis mutants. DACP, an excellent ethylene inhibitor, produced after the exposure to ultraviolet radiations has capacity for strong binding to the ethylene receptors when exposed continuously. However, it is explosive which limits its use (Sisler and Blankenship, 1993; Sisler *et al.*, 2006). There are some compounds from plant origin which have been found to have antagonistic effect such as some monoterpenes (Grichko *et al.*, 2003).

There was an immense amount of research work done in the past to assess the responses of horticultural commodities towards ethylene inhibitors. Both climacteric and non-climacteric paradigms have been used to explore the protecting effects of ethylene inhibitors on fresh produce spoilage (Watkins and Miller, 2006; Huber, 2008) and considerable modulation of the ripening related genes in both systems has been underpinned. It is reflected from earlier findings that remarkable responses of

climacteric fruits upon exposure to inhibitors are; changes in respiration rates and reduced ethylene biosynthesis, retention of firmness for longer periods of time, delay in colour changes, reduction or delay in the emission of volatiles (Rizzolo *et al.*, 2005; Menniti *et al.*, 2006; Zhang *et al.*, 2006; Ortiz *et al.*, 2006; Li *et al.*, 2006; Wang *et al.*, 2006). After the identification of ethylene's role in non-climacteric fruit development, some studies were carried out to reinforce the importance of ethylene antagonist to delay ripening and senescence in several non-climacteric fruits. Some examples in this context include delayed softening, colour development and ethylene production in strawberry (Bower *et al.*, 2003), suppressed levels of anthocyanin and berry size in intact grapes (Chervin *et al.*, 2004) and suppressed de-greening in grapefruit, 'Shamouti' orange and 'Tahiti' lime (Porat *et al.*, 1999; Jomori *et al.*, 2003; McCollum and Maul, 2007).

According to Goren *et al.* (2008) potent, water soluble, non-phytotoxic, non-volatile, odourless and broad-spectrum antagonists which are effective at very low concentration are required for practical use in the agriculture sector. Moreover, his study reveals that various inhibitors delay ripening in different fruits for varying periods of time depending upon molecular structure and concentration of inhibitors and the plant material used. Method of application also greatly affects the potency of inhibitors (Grichko *et al.*, 2005). Another important point for the practical use of ethylene inhibitors is the normal ripening of fruits after treatment with these chemicals (Sisler *et al.*, 2006).

Hence, there is a future need to develop water soluble and highly potent antagonist for ever growing horticulture sector which can easily cross the cell membranes as well as endoplasmic reticulum (ER) membranes where ethylene receptor (ETR1) is present. Therefore, they can be applied in a variety of plant systems in small concentrations without any restrictions of the sealed environment.

### **2.7.1. Cyclopropenes as Ethylene Antagonists**

Excessive use of toxic chemicals for postharvest treatments of food poses threats to human health and environment. According to World Health Organization (WHO), annually there are about 25 million cases of slow poisoning by the excessive use of chemicals in the food chain (Anonymous, 2002). Increasing health

consciousness of consumers and environment issues have shifted trends towards the use of bio based and other safe chemical formulations to enhance the shelf life of horticultural commodities (Asrey *et al.*, 2008). Cyclopropenes are the only ethylene inhibitors approved for application in edible crops as they are non-toxic to both humans and ecosystems (Grichko *et al.*, 2005). It has been confirmed by Environment Protection Agency (EPA, 2002) that they can be used in food crops without any harmful residues (Watkins, 2002; Blankenship and Dole 2003; Scariot *et al.*, 2014).

Cyclopropenes are organic compounds with a significantly high ring strain which makes them highly reactive (Rubin *et al.*, 2007). Ethylene inhibitors are known and have been used for more than 30 years but cyclopropenes and their derivatives are newly introduced (Serek *et al.*, 2007). Cyclopropenes and their alkyl derivatives have received significant attention in terms of their capacity to counteract ethylene responses (Reid and Staby, 2008; Halton, 2009). These compounds have been confirmed as the most effective ethylene antagonists as they form immensely stable adducts with ethylene receptor sites for longer period of times and irreversibly inhibit the ethylene action (Sisler *et al.*, 2006). In other words, they compete with ethylene for binding to receptors (Dupille and Sisler, 1995). The factors affecting the reactivity of cyclopropenes are their size, shape and substitution (Sisler *et al.* 2001). Some studies certify a direct relationship of molecular size to antagonistic effect and binding time (Grichko *et al.*, 2003). It is also hypothesized that compounds with higher molecular weight take more time to diffuse into the peel of fruits and may require higher concentration for protection. Two important parameters to determine the practical significance of cyclopropenes as demonstrated by Sisler *et al.* (2003) are minimum concentration required to control ethylene responses and maximum protection time. However, it is concluded from previous studies which have used a series of cyclopropenes, that concentration required for a specific compound increases when exposure time is reduced (Sisler *et al.*, 2003). Among ethylene inhibitors, cyclopropenes and STS are the only compounds which require a single exposure (Sisler *et al.*, 2006).

A range of cyclopropenes has been developed for ethylene action inhibition (Sisler and Serek, 2003). There are huge differences among the proficiency of cyclopropenes as ethylene inhibitors, as some of them bind to receptor sites for short

period of time while others are quite inactive. These compounds have competitive kinetics for receptors sites (Sisler and Serek, 1999). Ring strain of cyclopropenes is regarded as an important factor determining their receptor blocking capacity (Sisler and Yang, 1984). It has been proposed by earlier researchers that the strength of binding to metal receptor depends on ring strain. Hence, this factor led to the discovery of new antagonists with the passage of time as claimed by Sisler *et al.* (2003). Small ring structures have been proved to be more strained than larger ones (Wiberg, 1987). Some cyclopropenes like 1-MCP, CP and 3,3-DMCP were first to be tested as ethylene antagonists and have been shown to inhibit the ethylene responses by a single exposure for long-term basis i.e. 10-12 days at very low concentrations (Sisler *et al.*, 1996a). Afterwards, many other cyclopropenes were synthesized and used in scientific studies (Sisler *et al.*, 2003). Various cyclopropenes have substitution at 1, 2 and 3 carbon positions but those having methyl (CH<sub>3</sub>) at 1 position are more effective than others (Sisler *et al.*, 2001; Sisler *et al.*, 2003). 1-Alkyl-cyclopropenes are most effective ethylene inhibitors based on the previous studies (Sisler *et al.*, 1996b, 2003; Sisler and Serek, 1997). Some cyclopropene derivatives with varying chain lengths protected banana fruit for longer periods than 1-MCP. For example, 1-DCP delayed ripening in banana up to 36 days against higher ethylene levels at room temperature after single exposure of 24 hours (Sisler *et al.*, 2003). Another important point highlighted by this study was extremely low concentration (i.e. 0.3-0.5 nL<sup>-1</sup>) of longer chain cyclopropenes required to control ripening as compared to 1-MCP (i.e. 0.7 nL<sup>-1</sup>). Similarly, there are mono, di and tri substituted cyclopropenes e.g. 3-MCP, 1,2-DMCP, 1,3-DMCP, 3,3-DMCP, 1,3,3-TMCP, 3-methyl-3-vinylcyclopropene and 3-methyl-3-ethynylcyclopropene (Sisler *et al.*, 2001). Sisler *et al.* (2003) reported that although different cyclopropenes have different levels of activity and protection, 1-MCP is more useful from concentration point of view among mono, di and tri substituted compounds. In terms of practical use, 3,3 di substituted compounds are better as they are more stable.

Among cyclopropenes, 1-MCP is a commercially used synthetic plant growth regulator and a very potent ethylene action inhibitor (Serek *et al.*, 1995). Sisler and Serek (1997, 2003) have revealed the physical and chemical properties of 1-MCP including its mode of action. Further, various articles have reviewed its effect on quality parameters of harvested fruits and vegetables (Blankenship and Dole, 2003;

Watkins and Miller, 2006; Watkins, 2006; Huber, 2008; Watkins, 2008). It is apparent from these reviews as well as from other studies that until present, most of the studies have been done with 1-MCP to reduce the physiological and metabolic effects of ethylene in horticultural commodities as it is more active than other CPs (Watkins, 2008). This organic olefin has about 10 times more affinity for the ethylene receptor as compared to ethylene and binds at remarkably low doses (Blankenship and Dole, 2003). According to Sisler *et al.* (1996a), its effect is very prolonged at a very low concentration of  $0.5 \text{ nL}^{-1}$  as the treated bananas and tomatoes showed insensitivity towards ethylene for very long periods of time. Pure 1-MCP gas ( $15 \text{ mL}^{-1}$ ) can be used to treat  $30,000 \text{ m}^3$  space. Ethylene diffuses very quickly after its binding with receptor while 1-MCP remains bind for many days and does not allow ethylene to bind during this time (Sisler and Serek, 1997). It has been used to protect many fruits, vegetables, cut flowers and potted plants (Serek *et al.*, 1994; Mir *et al.*, 2001; Jiang and Joyce, 2002; Jeong *et al.*, 2003; Martinez-Romero *et al.*, 2003; Reid and Çelikel, 2008; Watkins, 2008; Seglie *et al.*, 2011; Razzaq *et al.*, 2016; Tucker *et al.*, 2017). The effective dose of 1-MCP varies for various crops (Watkins and Nock, 2005). It was patented in different countries for commercial treatment of fruits and vegetables including apples, bananas, melons and tomatoes by the trade name of Smartfresh<sup>®</sup> by Agrofresh, Inc. (Martinez-Romero *et al.*, 2007). Its use has been allowed in different countries for some other fruit crops including papaya, plums, avocados and persimmons (Sisler *et al.*, 2006) as EPA has confirmed its low animal toxicity based on experimental data (Reid and Staby, 2008). Fruits treated with 1-MCP ripe normally after a specific inhibition time period without loss of quality parameters (Argenta *et al.*, 2003). Temperature is an important factor determining its effective concentration and exposure time (Jiang *et al.*, 2004). There are certain drawbacks of 1-MCP which make its use difficult for commercial application. Its gaseous nature limits its application such as spraying and dip loading and its low water solubility and quick dissipation due to its volatile nature leads to false predictions about its effective concentration (Goren *et al.*, 2008). Recently, it is available as a water-soluble powder in commercial formulations having  $\alpha$ -cyclodextrin, which is stable in the dry form (Nanthachai *et al.*, 2007). This powder formulation has considerably increased the commercialization of 1-MCP by facilitating its storage and transport (Daly and Kourelis, 2001), apparent from its effective use in apple and pear crops (Elfving *et al.* 2007; Villalobos-Acuna *et al.*, 2010). There are some contradictory reports in this

context as Burns (2008) did not find promising results in citrus using sprayable 1-MCP. There is another concern regarding its application as pointed out by Vallejo and Beaudry (2006), that 1-MCP is absorbed/adsorbed by wood, cardboard and other materials in the cold storage facilities.

### 2.7.1.1. Mode of Action of Cyclopropenes

There are different theories and interpretations regarding the mode of action of cyclopropenes. Sisler *et al.* (1996a) and Dupille and Sisler (1995) presented their supposition that all the cyclopropenes bind to ethylene receptors similarly in a competitive manner and inactivate the receptor. Moreover, one of the hypotheses was that cyclopropenes may pull the metal out of the receptor to inactivate it. However, this concept did not seem right as different cyclopropenes have different time of inactivation, if metal is removed then time taken by plants to restore it would be the same for each cyclopropene. They further interpreted that different cyclopropenes bind with the receptor for different time periods and suggested that this difference might be due to varying vapour pressure of these compounds and hydrophobic interactions within the plant. Serek *et al.* (2007) speculated that prolonged effect of 1-substituted cyclopropenes with longer side chains (1-MCP to 1-DCP) may be referred to the hydrophobic side chains which anchor these inhibitors to the cell membrane causing a slow release effect. Both cyclopropene (CP) and 1-MCP are highly potent because of their excessive strain which cause them to bind tightly at receptor sites as concluded by Sisler *et al.* (1996a). These compounds have been shown to be 1000 times more active than 3,3-DMCP. Differential activity of 1-MCP and 3,3-DMCP is related to differences in their molecular structure i.e. steric factors.

Many research notes regarding the action of cyclopropenes indicate that hydrophobicity is an important criterion to determine the activity of these compounds as proposed by Serek *et al.* (2007). It was also asserted by Sisler *et al.* (2006) that less hydrophobic compounds with more water solubility are not efficient in antagonistic activity e.g. 1-cyclopropene methanol, when used in banana fruit, it only protected the peel not the pulp. Later, longer chain alcohol cyclopropenes were tested which counteracted the ethylene responses in whole banana fruit. Sisler *et al.* (2006) implied that hydrophobicity is crucial to reduce the water solubility of ethylene antagonists so that they can bind to the receptor sites which are located in the hydrophobic region.

The following figure (2.7) demonstrates the reaction between cyclopropenes and ethylene receptors as described by Pirrung *et al.* (2008).

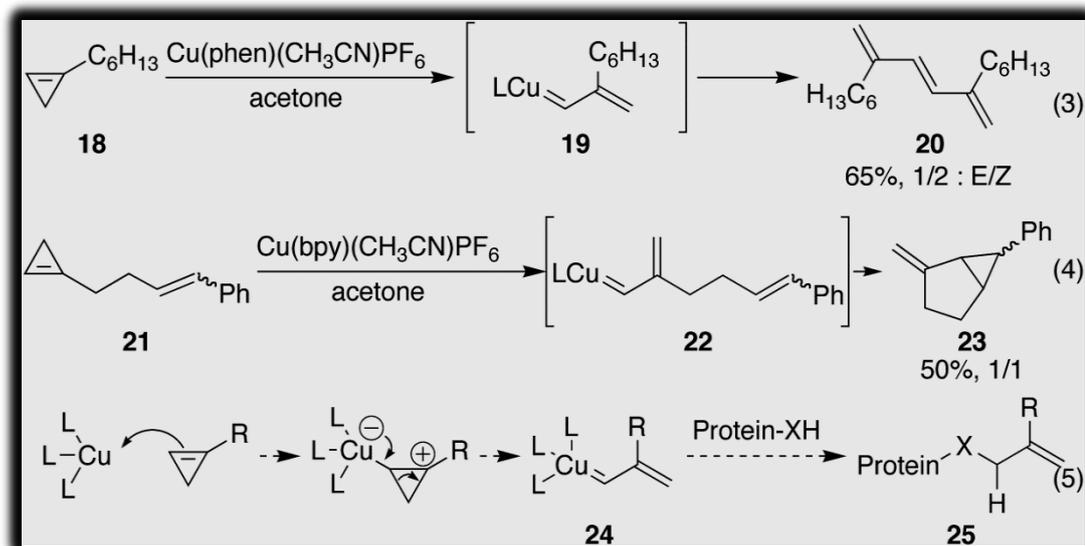


Figure 2.7. Model for the action of cyclopropenes to inhibit ethylene action (Pirrung *et al.*, 2008).

The copper ion ( $\text{Cu}^{+2}$ ) cofactor in the ethylene receptor could react with the 1-alkylcyclopropene to form a carbenoid (24). This intermediate is sequestered deep in the protein and reacts covalently with neighbouring protein residues and results in an inactive receptor (25). The long period of insensitivity of plant tissues to ethylene could be attributed to irreversible damage of the ethylene receptor which requires it to be replaced. Regaining sensitivity to ethylene by plant tissues would probably require the biosynthesis of new receptors.

Hoerberichts *et al.* (2002) reported a decline in mRNA abundance of genes which are concerned with ripening processes in tomato i.e. ACC oxidase 1, expansin 1 (EXP1) and phytoene synthase 1 (PSY1) by 1-MCP. Similarly, it affected the ACC accumulation and activity of ACC oxidase in banana fruit (Pathak *et al.*, 2003). Activity of some enzymes, such as chlorophyllase (CHLase) and peroxidase (POD) was also reduced by it as publicized by Gong and Mattheis (2003).  $\alpha$ -D-galactosidase,  $\beta$ -D-galactosidase,  $\alpha$ -D-mannosidase, and  $\alpha$ -D-glucosidase activities were also changed after 1-MCP treatment in apricots (Botondi *et al.*, 2003). Ethylene induced

activity of pectin methyl esterase (PME), polygalacturonase (PG), pectate lyase (PEL) and cellulase was declined by the application of cyclopropenes in banana (Lohani *et al.*, 2004). Further studies showed a decline in  $\beta$ -amylase activity, protein accumulation in banana (do Nascimento *et al.*, 2006) and reduced  $\beta$ -galactosidase and PME activities upon treatment with 1-MCP (Ortiz *et al.*, 2005).

### 2.7.2. Application of Cyclopropenes in Fruit Crops

Cyclopropenes and their substituted derivatives are a comparatively new introduction in postharvest technology, hence there is not much work regarding the effect of cyclopropene derivatives on harvested fruits. Most of the research trials have been done by Edward Sisler and his co-workers. As far as 1-MCP is concerned, there has been a huge amount of research carried out to evaluate its effect on pre and postharvest quality of fruits, vegetables and ornamentals. Some of the work in this context is reviewed here and summary for the application of ethylene antagonists to extend shelf life and to improve fruit quality is given in Table 2.7.

#### 2.7.2.1. 1-MCP

1-MCP was first synthesized by Fisher and Applequist (1965). History of 1-MCP as reviewed by Reid and Staby (2008) shows that detailed studies on CA storage and ethylene receptors has led to the discovery of this potent compound. Previous literature suggests that application of 1-MCP has considerably extended the shelf life of fruits by positively influencing the physiological and biochemical parameters. Rates of ethylene production, respiration and ripening were delayed in ‘Anjou’ pear, banana, apricot, ‘Angelino’ plum, papaya, ‘Golden Berry’ and ‘Golden Delicious’ apple, avocado, mango and custard apple (Golding *et al.*, 1998; Jiang *et al.*, 1999; Hofman *et al.*, 2001; Spotts *et al.*, 2007; Gutierrez *et al.*, 2008; Tucker *et al.*, 2017). Loss in weight and change in fruit colour was also slowed down in avocado (*Persea americana* Mill.), ‘West Indian’ lime (*Citrus aurantifolia*, Swingle) and banana (Jeong *et al.*, 2003; Win *et al.*, 2006). DeEll *et al.* (2005), Trinchero *et al.* (2004) and Harima *et al.* (2003) reported retention of firmness in ‘Empire’ apple, ‘Bartlett’ pear and persimmon (*Diospyros kaki* L.) cultivars respectively with 1-MCP. The positive role of this antagonist in the control of pathogenesis has been proved by previous studies as it protected the pear fruit from cell wall degradation enzymes of pathogens

(Spotts *et al.*, 2007). It also affected fruit development processes in non-climacteric fruits, for example; Porat *et al.* (1999) and Kluge *et al.* (2003) observed inhibition of de-greening in citrus and lime respectively by 1-MCP application. Total soluble solids (TSS) and titratable acidity (TA) in apples cv. 'Gala', 'Delicious', 'Granny Smith', and 'Fuji' were analysed in response to 1-MCP treatment, which delayed the loss of TA in all the four cultivars; while TSS was maintained in 'Gala' only (Bai *et al.*, 2005). Some physical and biochemical quality attributes were also maintained by 1-MCP in slices of 'Braeburn' and 'Pacific Rose' apples and in processed pineapple fruit (Perera *et al.*, 2003; Buda and Joyce, 2003). Similarly, vitamin C content in guava 'Allahabad Safeda' was positively affected by the combination of 1-MCP treatment and CA storage (Singh and Pal, 2008). Martinez-Romero *et al.* (2003) and Valero *et al.* (2003 and 2004) reported delay of postharvest changes during storage in early 'Golden Japan' and 'Santa Rosa' and late season plums cv. 'President', 'Reina Claudia' and 'Sungold' following 1-MCP application. Watkins and Miller (2006) have reviewed in detail the delay or decrease in postharvest metabolic changes and physiological disorders in fruits and vegetables using 1-MCP. Shaham *et al.* (2003) stated that considerable increase in antioxidant levels in the peel of apple fruit induced by 1-MCP resulted in protection against certain disorders e.g. superficial scald. Moreover, postharvest use of 1-MCP in apple cold storage eliminates the need for diphenylamine (DPA) application for the control of scald, which has toxic residual effects (Halton, 2009). Tonutti *et al.* (2007) evaluated the rate of ethylene production and fruit firmness in peaches cv. 'Summer Rich' and grapes cv. 'Raboso Piavea' following treatment with  $1 \mu\text{L}^{-1}$  1-MCP at 20 °C. Although the treated fruits were firmer than untreated, the effect of 1-MCP was not pronounced. Ethylene production rate was much less even after 3 days. However, it is not effective in every case, for example, chilling injury, off flavour and uneven colour changes have been observed in banana after 1-MCP treatment (Golding *et al.*, 1998; Jiang *et al.*, 2004).

A few research reports show that 1-MCP is ineffective for some crops. DeLong *et al.* (2004) reported that it had no effect on the storage life of blueberries and a very minute effect on strawberry has been reported by Bower *et al.* (2003). Similarly, it merely inhibited ethylene responses in stone fruit species (Argenta *et al.*, 2003) and partially ripe bananas (Pelayo *et al.*, 2003). The level of lactones was also decreased in apricots fumigated with 1-MCP (Botondi *et al.* 2003). Moreover, an increase in

physiological disorders and microbial infection has been reported in apple by Janisiewicz *et al.* (2003). Some studies documented browning and woolly breakdown in apple fruit using 1-MCP during storage (Dong *et al.*, 2002). Differential responses of different fruits to 1-MCP depend upon its concentration and treatment time as well as physical environmental conditions (Sisler *et al.*, 2006). More than optimum doses of 1-MCP delayed ripening in ‘Red Clapp’ and ‘Bartlett’ pear for too long with retardation of some ripening attributes (Ekman *et al.*, 2004; Calvo and Sozzi, 2004). Sometimes, its ineffectiveness is relevant to its instable nature or very low residual effects but now this problem has been resolved by the development of a specific device which releases the gas in a sustainable manner (Macnish *et al.*, 2004).

Cao and Zheng (2010) investigated the effect of 1-MCP on the incidence of anthracnose in loquat fruit during postharvest storage to replace the need for fungicides application. 1-MCP not only reduced the disease occurrence by increasing the activity of defence enzymes (chitinase and  $\beta$ -1,3-glucanase) but it also reduced the accumulation of reactive oxygen species (ROS) and increased the content of free radical scavenging enzymes i.e. SOD (superoxide dismutase), CAT (catalase), APX (ascorbate peroxidase). In addition, it maintained the various quality attributes throughout storage including TSS, TA and juice percentage.

Some researchers outlined the effect of 1-MCP in fresh cut slices of different fruits where it delayed softening, browning and microbial degradation. Vilas-Boas and Kader (2007) used 1-MCP to maintain firmness in fresh cut mango (cv. ‘Kent’ and ‘Keitt’), kiwifruit (cv. ‘Hayward’) and persimmon (cv. ‘Fuyu’) slices and concluded that it is effective in delaying softening and colour changes in all these fruits. Previously, an extended shelf life and inhibited loss of quality in slices of banana (Vilas-Boas and Kader, 2006), apple (Jiang and Joyce, 2002; Perera *et al.*, 2003; Bai *et al.*, 2005; Calderon-Lopez *et al.*, 2005), pineapple (Buda and Joyce, 2003) and papaya (Ergun *et al.*, 2006) has been added in literature. Pashazadeh *et al.* (2016) sprayed  $1\mu\text{L}^{-1}$  1-MCP on two cultivars of apple viz., ‘Wealthy’ and ‘Dirras-e-Mashhad’ and found it is effective for various quality factors like TA, pH and TSS after prolonged cold storage at 0.5 °C and 90 % RH. 1-MCP has been extensively used in apple crops on a commercial scale in different countries of the world but some cultivars do not respond well (Watkins, 2008; Watkins and Nock, 2012).

### 2.7.2.2. Cyclopropene (CP)

CP is a small planar molecule with high strain energy. It is a very reactive ethylene antagonist which has been used on a limited basis as it is very unstable (even at -80 °C) and has a danger of explosion at higher temperatures (Schipperijn and Smael, 1973; Sisler *et al.*, 2006). It must be stored at low temperature (-196 °C) or at room temperature (RT) under nitrogen without solvent because it decays in solvent at room temperature (Hopf *et al.*, 1985; Sisler and Serek, 1997). It protected the banana fruit (*Musa sapientum* L.) from adverse effects of high ethylene levels for a period of 12 days at the concentration of 0.7 nL<sup>-1</sup> (gas), which is just like that as reported for 1-MCP (Sisler *et al.*, 2001). Previously, it also controlled ethylene responses in banana at 0.5-0.7 nL<sup>-1</sup> while 0.4 nL<sup>-1</sup> proved ineffective (Sisler *et al.*, 1996b). Its level of activity is parallel to 1-MCP, but 1000 times more reactive than 3,3-DMCP (Sisler and Serek, 1997).

### 2.7.2.3. 3-Methylcyclopropene (3-MCP)

3-Methylcyclopropene is an isomer of 1-MCP but it has different biological activity and physical properties. According to Sisler *et al.* (2001) effective concentration and exposure time of cyclopropenes is influenced by steric and electronic effects of molecules. In addition, 3-MCP is a little bit less stable than 1-MCP and requires 5-10 times higher concentration for effective inhibition of ethylene action. It has been confirmed by different trials that 12 days insensitivity to ethylene (333 µL<sup>-1</sup>) is induced in banana at 2 nL<sup>-1</sup> (gas) 3-MCP compared to 0.7 nL<sup>-1</sup> for 1-MCP. However, the time of protection in banana fruit was the same i.e. 12 days (Sisler *et al.*, 2001, 2003, 2006). It counteracted ethylene responses in banana at 3.5 nL<sup>-1</sup> against 50 µL<sup>-1</sup> ethylene; while at 20 nL<sup>-1</sup> concentration, it protected fruits against 1000 µL<sup>-1</sup> of ethylene (Sisler and Serek, 1999)

### 2.7.2.4. 3,3-Dimethylcyclopropene (DMCP)

3,3-Dimethylcyclopropene is a quite stable compound even at higher temperatures in gas as well as in liquid state with a boiling point of 14.5 °C but it is less potent than 1-MCP and ethylene inhibition time is also short at a very high concentration as evidenced by a research study on banana fruit. It was effective at 500 nL<sup>-1</sup> (gaseous state) to protect banana fruit only for 7 days (Sisler *et al.*, 2001). Sisler

*et al.* (1996b) indicates  $1 \mu\text{L}^{-1}$  as an effective concentration of 3,3-DMCP. According to Sisler *et al.* (2006), more than 1 substituent at 3 position reduces the antagonistic capacity of cyclopropenes. Low reactivity level of 3,3-DMCP has been justified in a previously published article of Sisler and Serek (1997) which demonstrated that 2 methyl groups in its structure cause higher steric hindrance. These factors contribute to lowering its potency.

### 2.7.2.5. 1-Alkylcyclopropenes

Sisler *et al.*, (2003) synthesized a series of cyclopropenes substituted with side chains at 1 carbon position having 1-10 carbons (1-methyl to 1-decylcyclopropene). He found that the proficiency of cyclopropenes declined with increasing side chain lengths up to 4 carbons, however, it increased afterwards till 10 carbons even more than 1-MCP. Compounds with longer side chains proved to be more efficient in terms of concentration and time of protection from ethylene. It leads to a conclusion that cyclopropenes which are structurally large bind more strongly to ethylene receptors than smaller ones. All these compounds were in the gaseous phase and used for 24 hours treatment. Longer chain cyclopropenes i.e. 1-PentCP to 1-DCP were active to protect fruits at  $0.3\text{-}0.5 \text{ nL}^{-1}$  which is less than  $0.7 \text{ nL}^{-1}$  of 1-MCP.

There is an array of research-based studies which have been done to explore the potential of substituted cyclopropenes to manage ripening and produce quality with much focus on climacteric fruits including banana and avocado as evidenced by following literature. Xu *et al.* (2014) provided a testament for the proficiency of 1-pentylcyclopropene (1-PentCP). He treated bananas (*Musa acuminata* L. AAA group cv. Brazil) with  $0.4$ ,  $0.8$  and  $1.2 \mu\text{L}^{-1}$  1-PentCP in an air tight jar for 20 hours and stored them in ambient conditions for assessment. 1-PentCP ( $1.2 \mu\text{L}^{-1}$ ) yielded best results in terms of delaying ethylene and respiration rates, firmness and colour maintenance, and inhibited the expression of ripening related genes which are associated with ethylene production. Shun-Chang *et al.* (2013) tested  $2 \mu\text{L}^{-1}$  1-PentCP and  $0.5 \mu\text{L}^{-1}$  1-OCP (1-octylcyclopropene) in addition to  $0.75 \mu\text{L}^{-1}$  1-MCP to enhance the postharvest storage quality of apple cv. 'Hanfu' by treating them for 20 hours. They found 1-MCP and 1-PentCP effective to reduce ethylene production (by 8 days), respiration rate (by 4 days), loss of firmness and retarded an increase in TSS, melonaldehyde content and other degradative enzymes. On the other hand, 1-OCP

damaged the fruit quality by increasing the melonaldehyde content at the end of storage period. Previously, Goren *et al.* (2001) recommended the practical application of 1-ECP and 1-PCP for delaying fruit ripening in avocado after testing them on 'Hass' and 'Fuerte' for 24 hours following an exposure to 300  $\mu\text{L}^{-1}$  ethylene. The concentration used for 1-ECP was 0.5 and 1  $\mu\text{L}^{-1}$  for both cultivars which extended their life up to 10 days. In case of 1-PCP, it was 0.3 and 1  $\mu\text{L}^{-1}$  for 'Hass' while 1 and 1.5  $\mu\text{L}^{-1}$  was used for 'Fuerte' which led to an increase of 7 and 8 days respectively in shelf life. Feng *et al.* (2004) also verified the competency of 1-substituted cyclopropenes to delay ethylene induced ripening and senescence in same cultivars of avocado using 1-ethylcyclopropene (1-ECP) and 1-propylcyclopropene (1-PCP). Both these compounds were used in the form of gas in a sealed container at 22 °C for 24 hours and fruits were exposed to 300  $\mu\text{L}^{-1}$  ethylene afterwards. Different doses of these cyclopropenes were used to evaluate the optimum levels, and in both cases 1  $\mu\text{L}^{-1}$  was effective to delay the softening as well as colour changes. Overall results depict that 1-ECP was better in controlling ethylene responses than 1-PCP but not more than 1-MCP. Apelbaum *et al.* (2008) used the 'Hass' and 'Fuerte' cultivars of avocado to examine their response to 1-substituted cyclopropenes (*viz.*, 1-MCP, 1-ECP, 1-PCP, 1-BCP, 1-PentCP, 1-HCP, 1-HeptCP, 1-OCP, 1-DCP) by following the same experimental procedure as stated earlier by Feng *et al.* (2004). It was reinforced in this study that inhibitory effect of these compounds is highly influenced by their size and structure. 1-BCP and 1-PentCP were more potent as they delayed ripening by 12 and 11 days while softening was delayed by 18 and 17 days respectively when used at 500  $\text{nL}^{-1}$ . Rest of the compounds required very high concentration for ethylene inhibition i.e. 1000-1500  $\text{nL}^{-1}$ .

Other cyclopropenes substituted at 1, 2 and 3 carbon positions have been synthesized and used by Sisler *et al.* (2001). It is argued by authors that all the tested CPs vary greatly with reference to their level of activity which is remarkably determined by the number and position of methyl groups. 1,3,3-Trimethylcyclopropene (1,3,3-TMCP) and 1,2-dimethylcyclopropene (1,2-DMCP) required very high concentration i.e. 20,000 and 3000  $\text{nL}^{-1}$  and gave protection for 12 and 3 days respectively. These results suggest that 1,2-DMCP is less effective both concentration wise and short protection time.

### 2.7.2.6. Innovative Derivatives of Cyclopropenes

Goren *et al.* (2008) assessed the competency of 12 new CP derivatives with 1-position substitution and various side chains to control the ripening processes in climacteric fruits *viz.*, avocado, banana and peach. These derivatives were volatile and water soluble. These inhibitors proved to be more efficient than 1-MCP as ripening was delayed for about 3-12 days in fruits exposed to very high ethylene concentration after 24 hours pre-treatment with these new derivatives. In addition, a new water soluble CP derivative (WS-CPD) was synthesized from one of the new cyclopropenes *i.e.* 3-cyclopropyl-1-enyl-propanoic acid. All the tested fruits responded differently to this WS-CPD, as colour changes were delayed in avocado and banana while it did not markedly affect the firmness changes induced by ethylene. In peach fruit, the results were more encouraging probably due to the good penetration level by thin fruit skin.

### 2.7.2.7. Cyclopropene Salts: Novel Cyclopropene Derivatives

There are several limitations pertaining to effectual use of cyclopropenes like some of them are gaseous in nature which requires sealed environment and continuous exposure due to dissipation of gas. It also makes it difficult to calculate the final concentration received by plant material (Serek and Sisler, 2005; Serek *et al.*, 2007). Similarly, some formulations are highly affected by the ethylene concentration in the environment as well as by the treatment temperatures (Reid and Celikel, 2008; Seglie *et al.*, 2011). Furthermore, Sisler *et al.* (1996a and b) demonstrated the inefficiency of delivery vehicles of 1-MCP which are conventionally used. These vehicles require a very high input of active ingredients which pose many threats. Some of these drawbacks have been overcome by the use of advance chemical and postharvest technologies. For example, new formulations of 1-MCP have been synthesized which are non-volatile and can be applied without any restriction of sealed containers and risk of leakage. In addition, “Floralife” has released an innovative treatment system for 1-MCP by making tea bag like sachets which can be placed in water before treatment in any chamber to gradually and continuously release 1-MCP (Scariot *et al.*, 2014).

Another recent innovation is the synthesis of cyclopropene salts which have a methyl group at 1 carbon position where an amine group is substituted (Sisler *et al.*,

2009). These compounds have an advantage of dual chemistry as they can be used as a gas in sealed environment and as a salt (when mixed with weak acids) in open places. One example of cyclopropene salts is N, N-dipropyl-(1-cyclopropenylmethyl)amine (DPCA) (Sisler *et al.*, 2009; Seglie *et al.*, 2010). Sisler *et al.* (2010) assessed this cyclopropene salt to delay peel colour changes of banana fruit (*Musa paradisiaca*) at different doses of 0.2, 0.4, 0.8, 2.2, 3.3, 4.4, 8.8 nM for 24 hours both as a gas and liquid spray. The results were promising as it delayed the postharvest changes in response to ethylene for about 33 days. Although, DPCA gave good results in gaseous phase but it can be applied in outdoor systems as a salt (spray) with high feasibility. Previously, Sisler *et al.* (2009) appraised a series of dialkylamine compounds i.e. cyclopropene amine salts *viz.*, N,N-di-(1-cyclopropenylmethyl)amine (5.7 nL<sup>-1</sup>), N,N-dimethyl-(1-cyclopropenylmethyl)amine (73 nL<sup>-1</sup>), N,N-diethyl-(1-cyclopropenylmethyl)amine (59 nL<sup>-1</sup>), N,N-dipropyl-(1-cyclopropenylmethyl)amine (30 nL<sup>-1</sup>), N,N-dibutyl-(1-cyclopropenylmethyl)amine (184 nL<sup>-1</sup>), N-(1-cyclopropenylmethyl)aniline (248 nL<sup>-1</sup>). Treatment for 24 hours of banana fruit was done with these compounds both as a gas and salt followed by ethylene exposure as a standard. All these compounds inhibited ethylene responses for almost the same time period i.e. 32-34 days but concentration wise N,N-di-(1-cyclopropenylmethyl)amine and N,N-dipropyl-(1-cyclopropenylmethyl)amine were better than others. Moreover, exposure time and concentration was less for gaseous treatment than salts. There is a report on the use of another cyclopropene salt namely 3-cyclopropyl, 1-1-enylpropanoic acid sodium salt (CPAS) by Goren *et al.* (2011). It was applied in different modes including loading, dipping and spraying to retard ethylene responses in avocado cv. 'Hass', banana (*Musa acuminata* Colla.) cv. 'Grand Nanie' and peach (*Prunus persica*) cv. 'White Lady' for 30, 18 and 24 hours respectively. Concentration used was 0-500 mgL<sup>-1</sup> for avocado and banana while 0-200 mgL<sup>-1</sup> for peach. CPAS 100 mgL<sup>-1</sup> delayed ethylene effect in avocado for 9-13 days. In peach and banana, 200 mgL<sup>-1</sup> proved useful to delay ripening processes up to 18 and 4 days respectively.

With the passage of time, many new cyclopropenes have been prepared by adding different functional groups and substituting different side chains of varying lengths to revolutionize the postharvest technology. Choi (2013) has reported cyclopropene carboxylates for their inhibitory effects. Multiple 2-alkyl derivatives of 2-cyclopropene-1-carboxylic acid ethyl ester (alkyl CPEs) with 1-10 carbon chains

were synthesized and evaluated to reduce the rate of ethylene production and softening in ‘Taishuu’ and ‘Fuyu’ cultivars of persimmon. Fruit were treated with 20  $\mu\text{L}^{-1}$  of alkyl CPEs in gaseous phase and stored at room temperature for further analysis. The potency of CPEs extended directly with increasing carbon chain lengths. Hence, Decyl CPE (DCPE) appeared more beneficial in delaying softening and ethylene production. It is also useful for commercial application as it can be sprayed and more stable (Choi, 2013). There is another recent breakthrough in postharvest management of fruit ripening using dicyclopropene compounds as reported by Sisler and Grichko (2013). They observed 42 days delay in ripening of banana fruit with application of 1, 6-dicyclopropenyl-hexane at a very low concentration ( $0.3 \text{ nL}^{-1}$ ) when applied in the form of gas. It is a quite long-time protection reported using cyclopropene derivatives which can be tested in other fruit crops too.

### **2.7.3. Non-Conventional Ethylene Antagonists**

#### **2.7.3.1. Monoterpenes and other natural compounds as ethylene inhibitors**

Secondary plant metabolites have received much attention during last few decades to be used as plant-based growth regulators and pest control agents (Ibrahim *et al.*, 2004). Monoterpenes are a class of compounds which have 10-carbons and for which there are hundreds of examples (Grichko *et al.*, 2003). The major driving force behind an elevated use of these compounds is the consumer fear of human toxicity from synthetic chemicals and their residual effects including carcinogenicity and teratogenicity. Among these metabolites, monoterpenes are significant and known for least mammalian toxicity (Singh *et al.*, 2002). Secondary metabolites are known to be involved in the regulation of growth, transpiration and photosynthesis in plants (Banthorpe *et al.*, 1972). They are volatile compounds with specific aroma found in essential oils of plants. Archbold *et al.* (1997) reported that natural volatile compounds like 2-carene, D-limonene, 3-hexanone, can be used as a postharvest fumigant to prevent product loss. However, the mode of action of these compounds is not clear (Sharma and Pongener, 2010).

As far as the ethylene inhibition potential of monoterpenes is concerned, Grichko *et al.* (2003) documented a similarity in their structure with known ethylene inhibitors which suggests their capacity to compete for ethylene receptors and inhibit ethylene regulated processes. So, they tested different monoterpenes with varying

structures and inhibitor constant ( $k_i$ ) which was calculated by Line Weaver-Burk Plot (Burg and Burg, 1967). The tested monoterpenes include  $\alpha$ -terpinene,  $\gamma$ -terpinene, limonene,  $\alpha$ -isoprene,  $\beta$ -isoprene and carveol. All these monoterpenes proved weak ethylene inhibitors in comparison to cyclopropenes. The role of structure in ethylene inhibition could not be generalised because of huge structural diversity. However, compounds having *cis* and *trans* double bonds were more potent.

### 2.7.3.2. Limonene

Essential oils have been used in various research studies to prevent postharvest diseases of different fruits (Chebli *et al.*, 2004; Sharma and Tripathi, 2006). Limonene is an active component of some plant's essential oils like citrus (including *Citrus maxima* Burm. and *Citrus sinensis* (L.) Osbeck) and *Lippia scaberrima* Sond. Citrus oils are rich source of limonene (Lan-Phi *et al.*, 2009). Limonene has been used as antibacterial and anti-fungal agent to prevent infections of food by various species of microbes (Regnier *et al.*, 2008; Singh *et al.*, 2010). Use of antimicrobials from plant origin is gaining popularity in food industry due to increasing health concerns of consumers (Alzoreky and Nakahara, 2003). Regnier *et al.* (2008 and 2010) reported the use of limonene as a safer alternative to synthetic chemicals for the prevention of postharvest spoilage of horticultural commodities *viz.*, mango and avocado. Moreover, some Chinese scientist tested limonene for postharvest treatment of vegetables and fruits to maintain their quality and found promising results. For example, the influence of limonene emulsion on postharvest storage quality of green peppers and spinach was investigated by Lu *et al.* (2012 and 2013b). They reported that vitamin C, chlorophyll, soluble acids, soluble proteins and membrane permeability of these vegetables were maintained by limonene emulsion during storage. Similarly, Zhao and Yang (2014), Zhao and Shui (2014) and Zhao *et al.* (2015) assessed the impact of limonene emulsions on postharvest quality of apricot, nectarine and strawberry. They found a delayed loss of water and reduced percentage of rotting in strawberry by limonene treatment. In apricot and nectarine limonene inhibited the sharp changes in ascorbic acid, TA and TSS content. However, there are no reports of using limonene as an ethylene antagonist in horticulture crops to prevent deteriorating effects of ethylene. It has been reported by Solgi and Ghorbanpour (2014) that essential oil of *Lippia scaberrima*, which contains limonene, helps to maintain postharvest quality of fruits

like colour, firmness and aroma when used in commercial coatings. Espina *et al.* (2013) documented the bacterial inactivation by limonene and proposed its use in food industry for preservation purpose. The structural formula of limonene is shown in Figure 2.8.

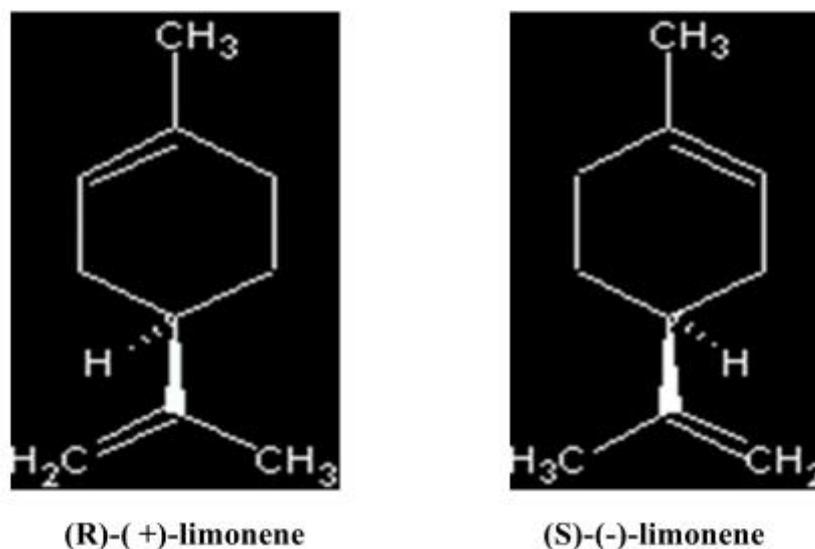


Figure 2.8. Structural formulas of limonene (Leffingwell, 2002).

### 2.7.3.3. Cinnamon Extract

An active component extracted from cinnamon oil has been identified as cinnamaldehyde which plays an important role to increase shelf life of horticultural produce as reported by Fujita *et al.* (2006) that it reduced the postharvest browning of lettuce. It is known as a GRAS (generally recognized as safe) chemical for food industry and it has shown an intense antimicrobial activity to prevent spoilage of fruits and to preserve their quality longer (Sanchez-Gonzalez *et al.*, 2011). High rate of fruit deterioration is associated with postharvest handling and storage particularly by fungal species of *Botrytis* and *Penicillium*. Cinnamon oil and its active components can effectively minimise the fungal rot due to their fungicidal potential (Combrinck *et al.*, 2011; Castillo *et al.*, 2012). Structural formula of *trans*-cinnamaldehyde is as shown in Figure 2.9.

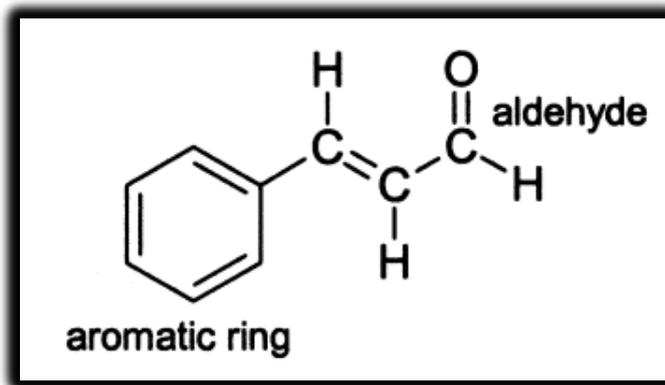


Figure 2.9. Structural formula of *trans*-cinnamaldehyde (www.sigmaaldrich.com).

According to Wendakoon and Sakaguchi (1995), cinnamaldehyde prevents the bacterial growth by inhibiting the activity of an important enzyme of cell metabolism i.e. amino acid decarboxylase. Moreover, cinnamon oil has a strong antioxidant activity which can play an important role in the defence mechanism against oxidative stress (Sanchez-Gonzalez *et al.*, 2011). There are various studies in which cinnamon oil or *trans*-cinnamaldehyde has been used to maintain quality and to prevent postharvest diseases of fruits and vegetables which are detailed below.

Cinnamon oil reduced the decay percentage and improved the quality of strawberry and tomato fruit in combination with eucalyptus oil (Tzortzakis and Economakis, 2007). In another study by Maqbool *et al.* (2011), anthracnose of banana and papaya was reduced remarkably by 0.4 % cinnamon oil when used with 10 % gum arabic in edible coating. Xing *et al.* (2011) also conducted a study to investigate the effect of cinnamon oil coating with chitosan on postharvest quality of sweet pepper after storage. Findings of their study showed that sensory quality of pepper was quite acceptable even after 35 days of storage at 8 °C. Moreover, the activity of antioxidant enzymes i.e. catalase, peroxidase and superoxide dismutase were higher in treated samples than control. Likewise, Carvalho *et al.* (2016) used *trans*-cinnamaldehyde for coating of fresh cut melons in combination with chitosan and found that it reduced the oxidative stress and improved the visual quality by inhibiting the browning enzymes. Structural integrity was also maintained well in cut melon (Figure 2.10). However, no reports have been found for the use of *trans*-cinnamaldehyde as an ethylene antagonist in postharvest studies.

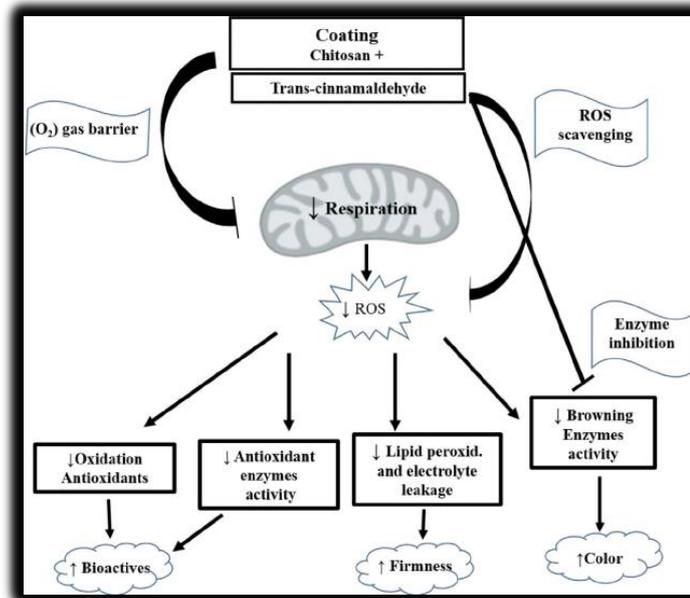


Figure 2.10. Mode of action and beneficial impact of *trans*-cinnamaldehyde on fruit quality with coating material (Carvalho *et al.*, 2016).

#### 2.7.4. Pome Fruit's Storage Techniques

Postharvest storage of fresh produce at favourable temperature and humidity helps to feed market demands for longer periods and minimize various losses of quality and quantity (Mahajan *et al.*, 2010). Generally, apple and pear have long periods of postharvest storage for year-round availability to markets. However, the quality attributes change to varying extents during storage due to biochemical and physiological changes, depending upon the cultivars (Costa *et al.*, 2012). Commonly employed storage techniques in the pome industry include cold storage/regular air (RA) storage and CA storage, which are reviewed below;

##### 2.7.4.1. Cold Storage

Low temperature during cold storage is an important factor which slows down the metabolic processes of fruit and ultimately retards the natural process of ripening (Ghafir *et al.*, 2009). Fruits under cold storage conditions also go through weight loss (Guleryuz *et al.*, 2000). In comparison to other fruits, apples have quite long storage life; however, there is a significant issue of fruit softness with prolonged storage which negatively affects the fruit quality and consumer acceptance (Kolniak-Ostek *et al.*,

2014). Moreover, low temperature also results in browning of pome fruits. Activity of polyphenol oxidase (PPO), a major enzyme responsible for browning of fruits, rises during cold storage (Saba and Moradi, 2016).

Changes in biochemical constituents and health benefit compounds of apple and pear have been reported during postharvest storage (Kou *et al.*, 2014; Lv *et al.*, 2016). For example, ascorbic acid, which is an essential vitamin from plant sources with antioxidant activity has been found to decrease in apples and pears during storage (Lee and Kader, 2000; Yazdani *et al.*, 2014). Similarly, polyphenols and other antioxidants of pome fruits go through changes during cold storage (Kevers *et al.*, 2011; Saba and Moradi, 2016).

Although, cold storage is one of the common practices to delay postharvest fruit deterioration, it is not always helpful to delay the physiological changes in pear as low temperature serve as a stimulant for ethylene production both pre and postharvest in both summer and winter pears. Chilling is a prerequisite to produce climacteric ethylene and fruit ripening. Growing season, variety, calcium concentration of the fruit and storage temperature are the factors which determine the storage period. For example, 'D' Anjou pears may need 30 or 60 days storage at -1 °C for climacteric ripening depending on the growth season (Gerasopoulos and Richardson, 1997). Similarly, in some apple cultivars low temperature during cold storage can cause some disorders like 'soft scald' or 'ribbon scald' in Honeycrisp apple, which is caused by storage below 2-3 °C and is characterised by brown spots on the skin and even flesh sometimes (Watkins *et al.*, 2005). One of major responsible factors for quality losses and other disorders during storage is ethylene production and perception by stored fruit and by inhibiting the ethylene action, storage life can be extended greatly (Yang *et al.*, 2013; Kolniak-Ostek *et al.*, 2014). Ethylene inhibitors have been used to maintain the sensory quality and nutritional attributes of apples and pear like 1-MCP which is most commonly used for successful storage (Tsantili *et al.*, 2007; Watkins, 2008; Kolniak-Ostek *et al.*, 2014). For instance, soft scald of 'Honeycrisp' apples after 6 months cold storage at 0-3 °C was minimized by 1-MCP application (DeEll and Ehsani-Moghaddam, 2010). Similarly, many other storage disorders of apples have been minimized by ethylene antagonists (Watkins and Nock, 2005; DeEll *et al.*, 2008). Consistent results have been reported in pears that the

application of 1-MCP maintained fruit firmness and other quality parameters while reducing weight loss after 75 days cold storage (Mahajan *et al.*, 2010).

### 2.7.4.2. CA Storage

Pome fruits are commonly stored in CA conditions for long term basis to extend the availability of these fruits by lowering O<sub>2</sub> and increasing CO<sub>2</sub> concentrations depending upon cultivars, which reduces the rate of climacteric ripening (Gwanpua *et al.*, 2012). Various studies have shown that CA helps to maintain fruit firmness, acidity, soluble solids and other nutrients in fruits and vegetables (Mattheis *et al.*, 2005; Thompson, 2010; Lee *et al.*, 2012; Calvo and Kupferman, 2012). Hence, this storage technique has led to the exploration of more markets worldwide by meeting the export standards (HAL, 2007; Gwanpua *et al.*, 2012).

However, CA storage is not always successful in keeping the fruit quality according to market criteria as there are various physiological disorders occurring during long term CA storage (Watkins and Nock, 2012). Core and flesh browning of apples and pears is one of the common disorders during storage caused by low temperature and high CO<sub>2</sub> concentration during CA (Saba and Moradi, 2016). Internal browning during CA storage is also accompanied with loss of fruit flavour, firmness and colour (Wang *et al.*, 2013). In addition, CA stored fruit sometimes fail to develop characteristic aroma (Lee *et al.*, 2012). Scientific evidence proved that treating pome fruits with ethylene antagonists before CA storage can effectively minimize the storage related issues (de Castro *et al.*, 2007; Park *et al.*, 2011)

DPA which is an antioxidant has been used to prevent storage diseases and disorders in apples and pears for almost 40 years, however, there are some serious concerns regarding the use of DPA which have been identified by European commission. Alternatively, 1-MCP is commonly used for successful storage of pome fruits and it has helped to prevent the storage scalds in apples effectively. In case of pears, 1-MCP application reduces storage and senescent scald but treated fruits fail to develop optimum ripening (Calvo and Kupferman, 2012).

In addition to the positive influence of 1-MCP on CA storage of pome fruits, some studies found its drastic impact on CA stored fruit. Fawbush *et al.* (2008) and

Argenta *et al.* (2010) have reported an increased occurrence of apple disorders due to CO<sub>2</sub> injury in 1-MCP treated fruit after CA storage. Similarly, incidence of flesh browning in CA stored 'Empire' apples is increased by 1-MCP (Jung and Watkins, 2011). These critical issues demand some new ethylene antagonist to address the shortcomings of 1-MCP.

Table 2.7. Summarized review for the application of synthetic and natural ethylene antagonists to maintain postharvest quality and extend storability of fruits.

Ethylene antagonists	Fruits/ cultivars	Mode of application	Application doses	Verdict	Reference
CP, 1MCP and 3,3-DMCP	Banana	Incubation for 24 hours	CP and 1- MCP @ 0.5 nL <sup>-1</sup> , 3,3 DMCP @ 500 nL <sup>-1</sup>	<ul style="list-style-type: none"> <li>• 1-MCP was more stable than CP and required in far less concentration than 3,3 DMCP.</li> <li>• Max time of insensitivity in banana was 12 days with CP &amp; MCP while 7 days with 3, 3 DMCP.</li> </ul>	Sisler and Serek (1997)
CP, 1-MCP, 3-MCP, 1,3-DMCP, 3,3-DMCP, 1,3,3-TMCP, 3-methyl-3-vinylcyclopropene, 3-methyl-3-ethynylcyclopropene and 1,2-dimethylcyclopropene.	Green bananas	24 hours treatment in sealed container	CP and 1-MCP @ 0.7 3MCP @ 2 3,3 DMCP @ 500 3-M-3-VCP @ 120 1,3,3-TMCP @ 20000, 3-M,3-ECP @ 240 1,3-DMCP @ 250, 1,2 DMCP @ 3000nL <sup>-1</sup>	<ul style="list-style-type: none"> <li>• All compounds blocked the ethylene receptor as bananas didn't respond to ethylene even at 1000 nL<sup>-1</sup>.</li> <li>• Ethylene responses were delayed for 12 days.</li> </ul>	Sisler <i>et al.</i> (2001)

CP, 1-Methyl-, 1-ethyl-, 1-propyl-, 1-butyl-, 1-pentyl-, 1-hexyl-, 1-heptyl-, 1-octyl-, 1-nonyl-, and 1-decylcyclopropene	Green bananas	24 hours exposure to CPs in a container. Low MW inhibitors were injected by syringe in container while, high MW pipetted out in ether on filter paper.	0.3-6.0 nL <sup>-1</sup>	<ul style="list-style-type: none"> <li>• 1DCP protected bananas for maximum time at ambient temperature i.e. 36 days.</li> </ul>	Sisler <i>et al.</i> (2003)
1-ECP and 1-PCP	Avocado cv. 'Hass' and 'Fuerte'	Incubation	1-ECP @ 0.1, 0.5, 1.0 and 2.0 μL <sup>-1</sup> while, 1-PCP @ 0.15, 0.3, 1.0 and 1.5 μL <sup>-1</sup>	<ul style="list-style-type: none"> <li>• 1-ECP was more effective to control ripening than 1-PCP but less than 1-MCP.</li> <li>• Max protection time was 13-14 days from softening and colour changes.</li> </ul>	Feng <i>et al.</i> (2004)
3-Cycloprop-1-en-1-ylpropan-1-ol, 1-(butoxymethyl) cyclopropene, 1-[(hexyloxy)methyl]cyclopropene, 1-cycloprop-1-en-1-ylmethanamine, Cycloprop-2-en-1 one, 2-methylcycloprop-2-en-1-one, 2,3-dimethylcycloprop-2-en-1-one, 2,3-diphenylcycloprop-2-en-1-one ,	Mature green banana	Incubation	Varied with compounds	<ul style="list-style-type: none"> <li>• Antagonistic activity of CPs declined with increasing MW and decreased water solubility.</li> <li>• Max protection time was 37 days by 1-nonylcyclopropene.</li> </ul>	Grichko (2006)

Cycloprop-2-ene-1-carboxylic acid,  
 2-methylcycloprop-2-ene-1 carboxylic  
 acid, 3-cycloprop-1-en-1-ylpropanoic  
 acid, Propan-2-aminium 3-cycloprop-1-  
 en-1-ylpropanoate,  
 2(aminomethyl)cycloprop-2-ene-1  
 carboxylic acid,  
 3(aminomethyl)cycloprop-1-ene-1-  
 carboxylic acid,  
 1-heptylcyclopropene, 1-  
 nonylcyclopropene, 1-decylcyclopropene,  
 3-methylcyclopropene, 3-  
 pentylcyclopropene , 3-  
 heptylcyclopropene and  
 1 MCP

12 volatile cyclopropene derivatives and  
 3-(cycloprop-1-enyl-propanoic acid,  
 sodium salt (CPAS)

Banana  
 avocado and  
 peach

Incubation in  
 sealed chambers  
 with volatile  
 compounds  
 While, CPAS  
 was applied as  
 Spray, loading  
 and brushing

CPAS @ 200  
 $\mu\text{g mL}^{-1}$  for  
 banana, 100  
 $\mu\text{g mL}^{-1}$ for  
 avocado and  
 10  $\mu\text{g mL}^{-1}$ for  
 peach

- Delayed ripening by 3-12 days by volatile compounds, while CPAS delayed peel colour changes in banana and avocado up to 6 days and retained firmness in peach up to 4 days. Goren *et al.* (2008)

1-ECP,1-PCP, 1-BCP, 1-PentCP, 1-HCP, 1-HeptCP, 1-OCP &1-DCP	Avocado cv. 'Hass' and 'Fuerte'	24 hours incubation in sealed drum	500-1500 nL <sup>-1</sup>	<ul style="list-style-type: none"> <li>1-HCP delayed ripening for max 12 days and 1-BCP was more effective with delayed ethylene peak upto 17 days and softening up to 18 days.at lower concentration.</li> </ul>	Apelbaum <i>et al.</i> (2008)
N,N-dialkyl-(1-cyclopropenylmethyl)amine compounds;	Bananas	24 hours incubation using as a gas and applied as a salt with a swab on banana peel	5.7 - 248 nL <sup>-1</sup>	<ul style="list-style-type: none"> <li>Maximum protection time from ethylene was 32-34 days.</li> </ul>	Sisler <i>et al.</i> (2009)
N,N-dipropyl(1-cyclopropenyl-methyl)amine (DPCA)	Banana	Loading, dipping spraying	Not reported	<ul style="list-style-type: none"> <li>33 days protection in banana against ethylene induced colour changes.</li> </ul>	Sisler <i>et al.</i> (2010)
CPAS	Avocado, banana, peach and citrus	Loading, dipping spraying	0 - 500 mgL <sup>-1</sup> for avocado and banana, 0-200 mgL <sup>-1</sup> for peach and 0-189 mgL <sup>-1</sup> for citrus	<ul style="list-style-type: none"> <li>Delayed colour change and softening in banana, avocado and peach while delayed abscission in citrus was recorded.</li> </ul>	Goren <i>et al.</i> (2011)

Cinnamon oil	Banana and papaya	As an edible coating with gum arabic	0.4 % cinnamon oil	<ul style="list-style-type: none"> <li>• It helped to control anthracnose by 70-80 % in both fruits.</li> </ul>	Maqbool <i>et al.</i> (2011)
1-MCP, 1-PentCP and 1-OCP	Apple cv. 'Hanfu'	Fumigation	1-MCP @ 0.75, 1-PentCP @ 2 and 1-OCP @ 0.5 $\mu\text{L}^{-1}$	<ul style="list-style-type: none"> <li>• 1-MCP and 1-PentCP reduced ethylene peak by 8 days and respiration peak by 4 days, delayed firmness loss, retarded changes in SSC, malondialdehyde and antioxidant contents.</li> <li>• 1-OCP increased malondialdehyde content causing adverse effect on storage.</li> </ul>	Cheng <i>et al.</i> (2012).
1,6-dicyclopropenyl-hexane	Banana	Gas treatment	0.3 nl L <sup>-1</sup>	<ul style="list-style-type: none"> <li>• 42 days protection from ethylene responses.</li> </ul>	Sisler and Grichko (2013)
Cyclopropene Carboxylate (alkyl CPEs) with alkyle chain length of 1-10 carbons.	Persimmon cv. 'Taishuu' and 'Fuyu'	24 hours incubation in sealed container	CPEs @ 20 $\mu\text{L}^{-1}$ and 1MCP @ 1 $\mu\text{L}^{-1}$	<ul style="list-style-type: none"> <li>• CPEs with long alkyle chains were most effective in delaying ripening parameters.</li> <li>• Particularly decyl CPE was stable and sprayable. It delayed postharvest</li> </ul>	Choi (2013)

1-PentCP	<i>Musa acuminata</i> L. AAA group cv. 'Brazil'	Incubation for 24 hours at room temp.	0.4-1.2 $\mu\text{L}^{-1}$	<p>flesh softening of persimmon fruit.</p> <ul style="list-style-type: none"> <li>1-PentCp was effective in delaying ethylene peaks and respiration rates, colour changes, softening, ACC content, ACC synthase and ACC oxidase enzymes activities.</li> </ul>	Xu <i>et al.</i> (2014)
Limonene	Strawberry apricot and nectarine	As an emulsion for 3 minutes dipping	Not reported	<ul style="list-style-type: none"> <li>Limonene retained freshness of fruit for longer and reduced the quality changes like colour, acidity, SSC and other nutritional values.</li> </ul>	Zhao and Yang (2014) Zhao and Shui (2014) Zhao <i>et al.</i> (2015)
1-MCP, 1-PentCP and 1-OCP	Hardy kiwifruit ( <i>Actinidia arguta</i> Miquel)	Incubation for 20 hours at room temp.	0.4-1.2 $\mu\text{L}^{-1}$	<ul style="list-style-type: none"> <li>1-OPC delayed ripening associated quality decline and maintained higher antioxidant activity better than 1-PentCP but slightly less than 1-MCP.</li> </ul>	Wang <i>et al.</i> (2015)
1-OCP	Tomato fruit	Incubation for 20 hours	0.4-1.2 $\mu\text{L}^{-1}$	<ul style="list-style-type: none"> <li>1-OCP effectively delayed the ripening</li> </ul>	Xu <i>et al.</i> (2016)

		at room temp.		related changes by inhibiting the activities of ethylene biosynthesis enzymes and ethylene expression genes.
1-HCP	Tomato fruit	18 hours incubation in sealed containers	500, 1000 and 2000 nL <sup>-1</sup>	<ul style="list-style-type: none"> <li>1-HCP delayed changes in colour, SSC, TA, suppressed ethylene and respiration rates and maintained higher organic acid contents.</li> </ul>
<i>trans</i> -Cinnamaldehyde	Melon, 'Ponkan' citrus and 'Satsuma' mandarin	As a component of edible coatings with chitosan	500 mgL <sup>-1</sup> 0.13-2.0 mL <sup>-1</sup> 0.5-2.0 mL <sup>-1</sup> respectively	<ul style="list-style-type: none"> <li><i>trans</i>-Cinnamaldehyde acted as a radical scavenger in fresh cut melon and improved its visual quality by inhibiting browning enzymes.</li> <li>In citrus, green mould decay and sour rot was significantly reduced.</li> </ul>

### 2.7.5. Conclusions

An overview of the literature regarding ethylene antagonists indicates that they control the ethylene action at molecular and physiological levels. Fruits are very complex parts of plants in terms of morphological, biochemical and physiological features. Highly variable responses of different fruits towards a specific ethylene antagonist can be referred to tissue specific variations in ethylene receptor attributes. Hence, further research is required to evaluate the cultivar specific responses towards various novel ethylene antagonists as well as to identify the differential selectivity of receptor sites for various compounds to standardize the protocols for long-term storage of horticultural commodities (Huber, 2008).

Moreover, previously used ethylene antagonists have various drawbacks which limit their full commercial potential. For example, heavy metal toxicity by Ag<sup>+</sup> ion and STS, pungent odour of 2,5-NBD and explosiveness of DACP (Sisler *et al.*, 2006; Reid and Staby, 2008). Similarly, 1-MCP which is commonly used in commercial fruit industry has some critical adverse effects on fruit quality and is highly expensive. Hence, there is a need to continue research to explore more potent compounds as ethylene antagonist, which can be commercially applied in diverse crops with cheap availability and long-term protection to horticultural commodities. In addition, an increasing awareness of consumers regarding health and environment safety demands search of new compounds which can be used without/minimal harmful residual effects (Asrey *et al.*, 2008). Therefore, new plant-based compounds warrant to be tested for their ethylene antagonistic potential to replace toxic synthetic formulations. Various secondary plant metabolites, monoterpenes and essential oils have been reported to positively affect the quality of fresh produce such as limonene and *trans*-cinnamaldehyde (Grichko *et al.*, 2003; Sharma and Pongener, 2010; Carvalho *et al.*, 2016). However, these compounds have not been used as ethylene antagonists previously and should be assessed for their potential to inhibit ethylene action in plants.



### 3.2. Experimental Site

Fruits were transported immediately after harvesting to the Horticulture Research Laboratory, School of Molecular and Life Sciences, Bentley Campus, Curtin University, Perth, where experiments were conducted. A random sample of fruits was selected to assess zero-day quality parameters such as fruit maturity, colour, firmness, titratable acidity (TA) and soluble solid content (SSC) to compare the influence of cold storage and ethylene antagonists at the end of storage periods.

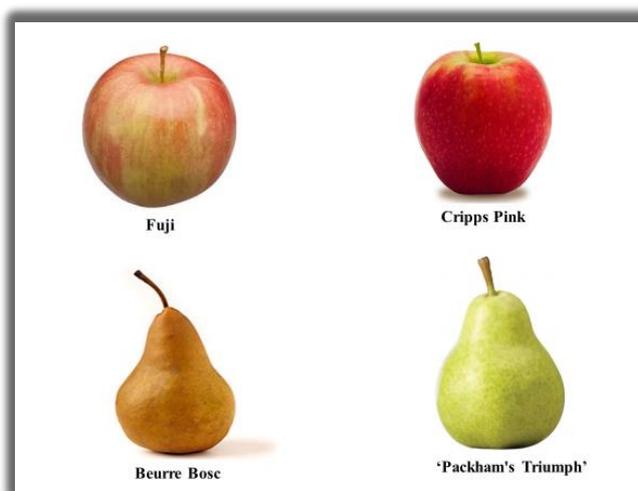


Figure 3.2. Experimental cultivars of apple and pear (APAL, 2018).

### 3.3. Assessment of Fruit Maturity

Iodine-starch test was conducted to check the maturity stage of apple fruit. However, in the case of pears, flesh firmness was used as a maturity index and fruit with  $<$  or  $>$  9.0 Kg/cm<sup>2</sup> firmness were used for experiments (APAL Maturity Standards, 2018).

#### 3.3.1. Iodine-Starch Test

##### 3.3.1.1. Preparation of iodine solution

To prepare iodine solution, 2 g potassium iodide (KI) was dissolved in 100 ml of distilled water by gentle stirring. Once KI was completely dissolved, 0.5 g of iodine crystals were added with continuous stirring until a brown colour solution was obtained.

##### 3.3.1.2 Testing procedure

A solution of iodine crystals and KI was used to check the starch staining patterns of apple fruit as a measure of fruit maturity stage. Fruit were cut half way through the equator and were placed on a thin layer of iodine solution for about 60 seconds. The test was performed within 24 hours after harvesting. In both ‘Fuji’ and ‘Cripps Pink’, flesh was 40-60 % stained as per the guide (Department of Primary Industries and Regional Development, Western Australia) indicating that fruit were not over-ripe nor under-ripe (Figure 3.3 and 3.4).

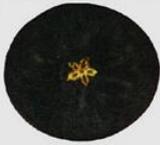
Marketing	Starch degradation (1–6 scale)	
Immature Do not harvest		<b>1:</b> Full black stain with no clearing in cortex.
Long term storage maturity		<b>2–3:</b> Clearing extends into the cortex (30%). Core partly clear.
Optimum storage maturity		<b>3–4:</b> Clearing extends into cortex (50–60%). Core clear.
Short term storage maturity		<b>4–5:</b> Clearing extends to 70% of cortex. Core clear.
Mature fruit Immediate sale		<b>6:</b> Almost entire cortex clear.

Figure 3.3. Starch staining pattern guide for apple (Department of Primary Industries and Regional Development, Western Australia, 2014).

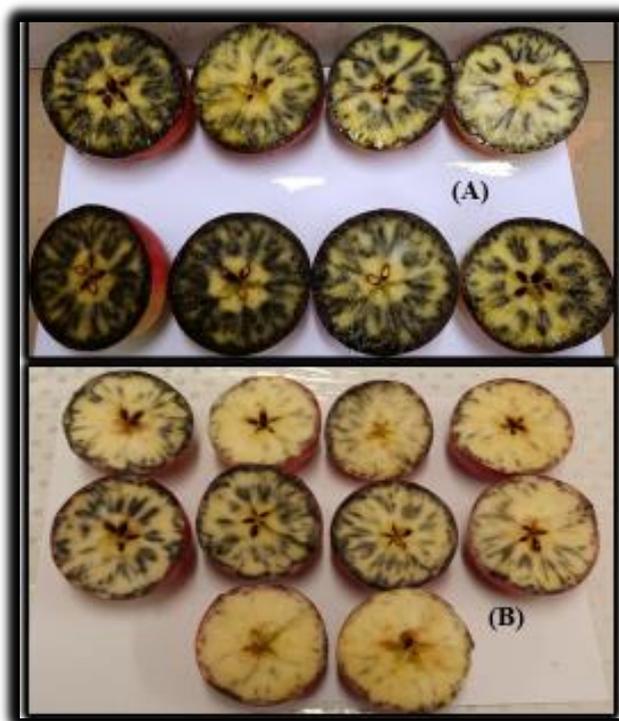
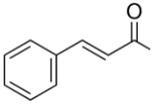
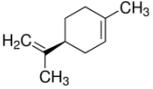


Figure 3.4. Starch staining patterns of (A) 'Fuji' fruit harvested on 15th April, 2015 and (B) 'Cripps Pink' harvested on 8<sup>th</sup> May, 2015.

### 3.4. Fumigation with Ethylene Antagonists

Uniform sized apple and pear fruits were selected for treatment with 1-MCP and some novel ethylene antagonists (Table 3.1). Fumigation of fruits was done with 1  $\mu$ M solution of different ethylene antagonists in an airtight plastic canister for 24 hours. Solutions of ethylene antagonists (5 ml) were placed inside the container in a small Petri dish with a battery operated fan for equal dissemination of chemical fumes and soda lime (25 g) to absorb excessive CO<sub>2</sub> (Figure 3.5). For 1-MCP, tablets of commercial formulations from 'AgroFresh Solutions' were used which provided 740 parts per billion (ppb) of 1-MCP.

Table 3.1. Chemical names, structural and chemical formulas, molecular weights and sources of ethylene antagonists.

Chemical Name	Chemical Formula	Structure	MW (g)	Source
1-Methylcyclopropene	C <sub>4</sub> H <sub>6</sub>		54.09	AgroFresh Solutions (AGFS), USA
1-Hexylcyclopropene	C <sub>9</sub> H <sub>16</sub>		124	Payne's research group, Chemistry Dept. Curtin
<i>trans</i> -Cinnamaldehyde	C <sub>9</sub> H <sub>8</sub> O		132.2	ACROS Organic (Thermo Fisher Scientific, Victoria, Australia)
( <i>S</i> )-(-)-Limonene	C <sub>10</sub> H <sub>16</sub>		136.2	ACROS Organic (Thermo Fisher Scientific, Victoria, Australia)

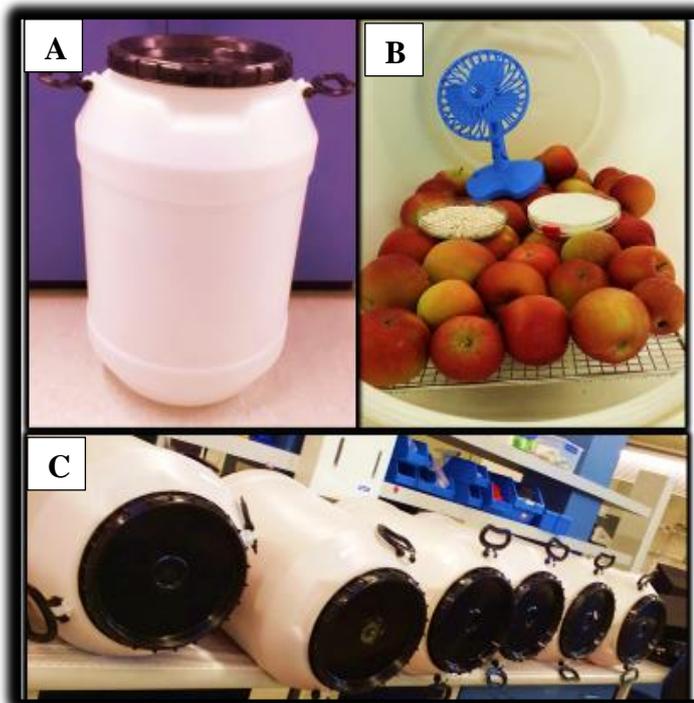


Figure 3.5. An illustration of the fruit treatment with ethylene antagonists: (A) Hermetically sealed plastic containers of 60 L volume (B) Setup for fumigation of apple fruit with ethylene antagonist's chemical solution with soda lime and small fan (C) Plastic containers sealed for 24 hours fumigation treatment.

### 3.5. Cold Storage Conditions

Following the treatment, the apple fruit were stored at  $0.5\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$  temperature and  $85 \pm 5\%$  relative humidity (R.H.) in cold rooms of Horticulture Research Laboratory at Curtin, Bentley Campus. Cold storage for pear fruit was adjusted with  $0 - 1\text{ }^{\circ}\text{C}$  temperature and  $> 85 \pm 5\%$  R.H. Apple fruit were cold stored for the period of 28, 75 and 120 days while pear fruit were stored in cold rooms for 4 and 6 months. Following each cold storage period, fruit were shifted to simulated shelf conditions ( $21 \pm 1\text{ }^{\circ}\text{C}$ ) and ethylene production and respiration rate were monitored daily until post climacteric peaks were noted. Firmness, colour, SSC, TA, SSC/TA ratio, ascorbic acid, total phenolics, total antioxidants, individual and total sugars, as well as organic acids on 10<sup>th</sup> day of simulated shelf conditions were assessed. Temperature and R.H. was monitored during cold storage periods using Tinytags

(Gemini Data Loggers, West Sussex, UK) run by Tinytag Explorer Software (Figure 3.6 and 3.7).

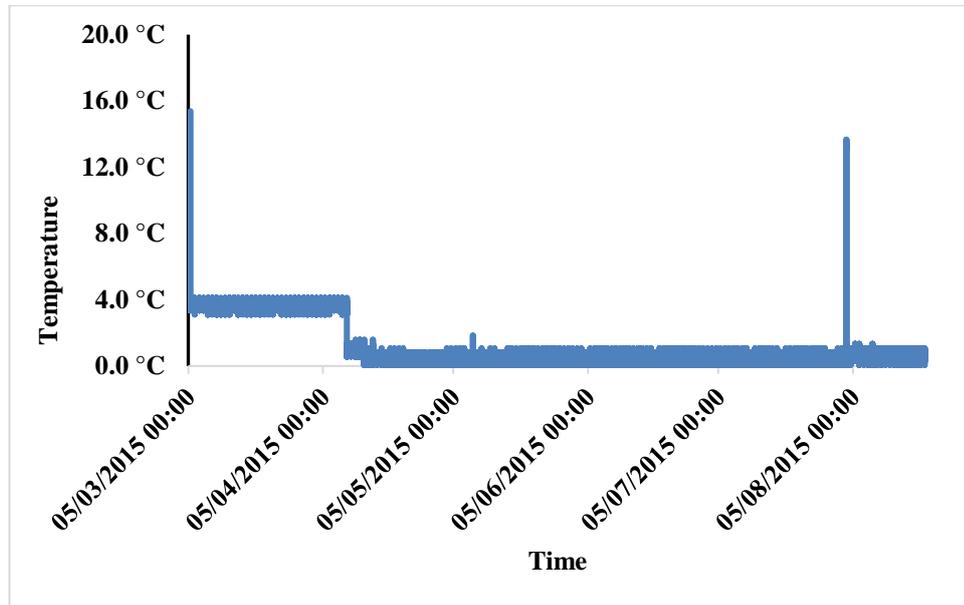


Figure 3.6. Temperature data recorded during cold storage period of fruit using Tinytag data loggers.

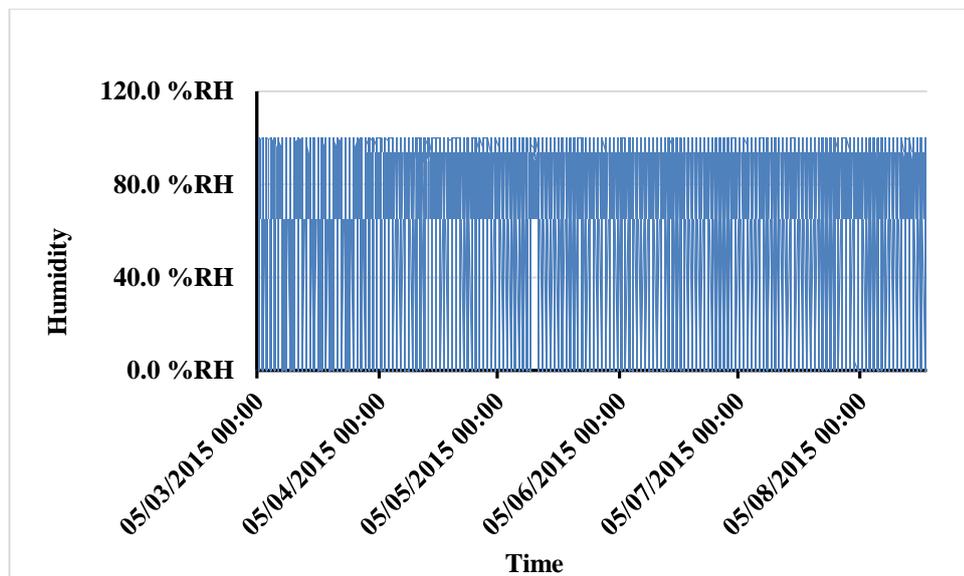


Figure 3.7. Relative humidity recorded during cold storage period using Tinytag data loggers.

### 3.6. CA Storage Conditions

After treatment of apple fruit with ethylene antagonist for 24 hours, both treated and untreated (control) fruit were stored in CA storage rooms in the property of Newton Brothers at Manjimup. Storage conditions in CA rooms were maintained with 2 % O<sub>2</sub> and 0.5 % CO<sub>2</sub> at 1 °C with 95 ± 5 % RH. Fruit were stored in CA for a period of 6 and 8 months depending upon cultivar.

### 3.7. Observation Recorded

Ethylene antagonist's  
Soln.

#### 3.7.1. Ethylene Production

Ethylene production rate from pome fruits was determined using a laser-based system (ETD-300; Sensor Sense B.V., Nijmegen, The Netherlands) (Figure 3.8) as elaborated previously by Cristescu *et al.* (2013). Two uniform sized fruit from each experimental unit without any bruises were selected for estimation of ethylene production rate. After weighing the fruit samples, they were placed inside cuvettes of 1L volume, sealed with a rubber septum and connected to an ethylene detector. To avoid the build-up of pressure in cuvettes, output channels were checked for any blockage before connecting flow to the ethylene detector. Sample running time was 20 min and flow rate was adjusted to 4 L per hour. Rate of ethylene production was expressed as pmol kg<sup>-1</sup> s<sup>-1</sup>.

#### 3.7.2. Respiration Rate

For estimating respiration rate, fruits with uniform weight and colour from each experimental unit were kept in sealed glass jars of 1L volume fitted with a rubber septum (SubaSeal®, Sigma-Aldrich Co., St. Louis, USA) at room temperature. After 1-2 hours incubation of fruits, headspace gas samples were used to estimate CO<sub>2</sub> concentration by using an infrared gas analyser (Servomex Series 1400, Sussex, UK) for the determination of respiration rate (Figure 3.9) and expressed as μmol kg<sup>-1</sup> s<sup>-1</sup>.

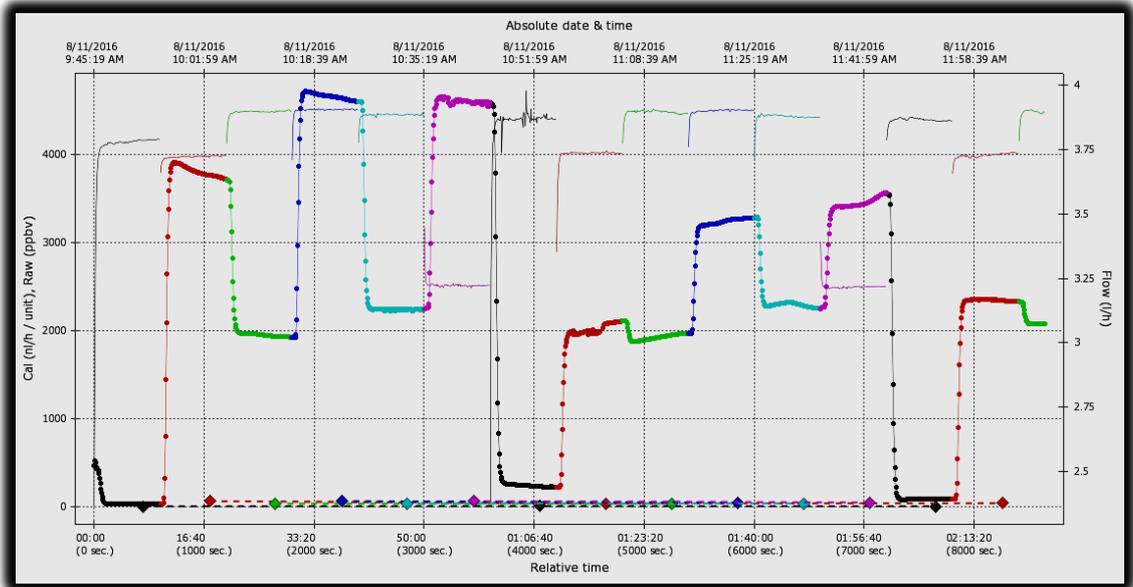


Figure 3.8. Estimation of ethylene production rate from fruit samples by Sensor Sense.

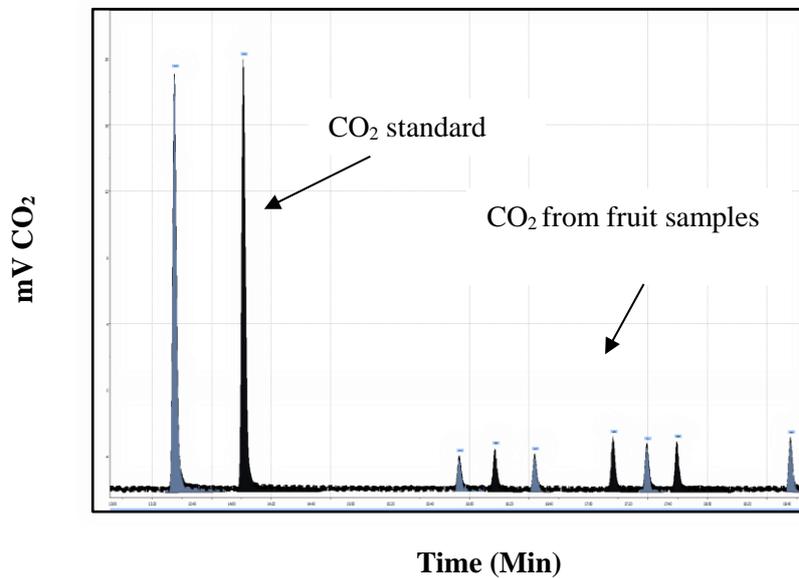


Figure 3.9. Chromatograph of CO<sub>2</sub> standard and headspace gas samples of fruits from infra red gas analyser.

### 3.7.3. TA, SSC and SSC/TA Ratio

In order to measure TA, 10 ml mixture of juice from 20 fruit in each replication was diluted with 20 ml distilled water (d.H<sub>2</sub>O). Following the dilution, 5 ml aliquot was titrated against 0.1N NaOH. Phenolphthalein (3,3-bis(4-hydroxyphenyl)-2-benzofuran-1(3H)-one) was used to indicate the end point of titration by turning juice colour to pink. Acidity was expressed as malic acid equivalents (%) and was calculated by the given formula.

$$\text{TA (\%)} = \frac{\text{Equivalent factor for malic acid (0.0067)} \times \text{volume of titrant} \times 100}{\text{Volume of juice (ml)} \times \text{volume of aliquot (ml)}}$$

A battery-operated refractometer (ATAGO™ Digital Palette Refractometer, PR-101, Tokyo, Japan) calibrated at 20 °C was used to determine SSC from juice samples of each replicate and expressed as a percentage. For the calculation of SSC/TA ratio, the values of SSC (%) were divided by the corresponding TA (%) values.

### 3.7.4. Fruit Firmness

Firmness was determined as a resistance to penetration on two opposite equatorial sides of fruit by means of a texture analyser (TA Plus, Lloyds Instruments, Hampshire, UK) fitted with 11.1 mm diameter plunger and operated by Nexygen™ Plus Material Testing Software (Figure 3.10). Fruit firmness was expressed in Newtons (N).

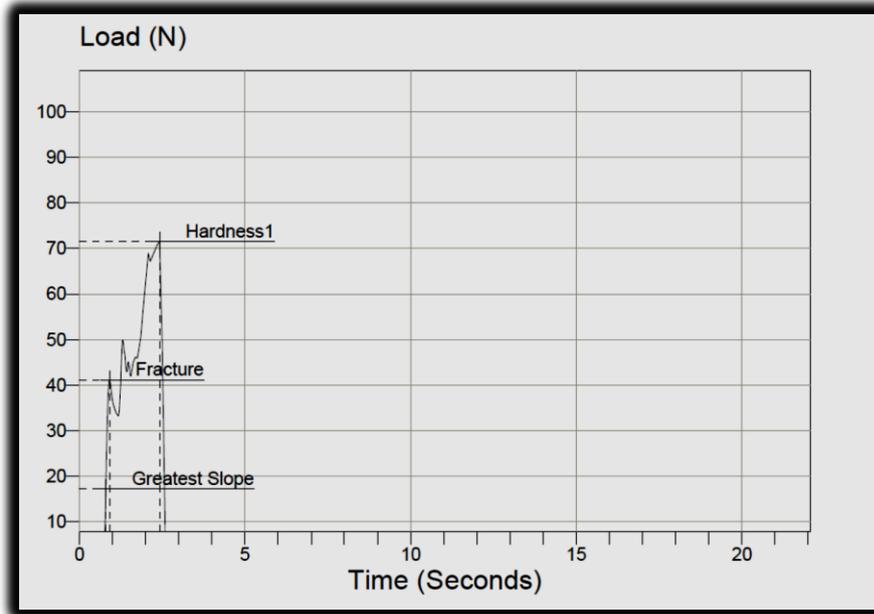


Figure 3.10. Analysis of fruit firmness using Texture Analyser and Nexygen™ software.

### 3.7.5. Fruit Colour

Peel colour of apple and pear was recorded at diametrically two opposite sides of fruit using a ColorFlex 45°/0° Spectrophotometer (Hunter Associates Inc., Reston, VA, USA) as  $L^*$ ,  $a^*$  and  $b^*$  (Figure 3.11 and 3.12).  $L^*$  values refer to lightness in fruit colour and measured from 0-100 i.e. black to white.  $a^*$  values are the measure of red and green colour whereas,  $b^*$  reflects yellow or bluish colour of the fruit skin (Rehman *et al.*, 2018). Hue angle ( $h^\circ$ ) represents the changes in fruit colour with progress of ripening events and is exhibited by a line from the origin to the intercept of X ( $a^*$ ) and Y ( $b^*$ ) axis on colour wheel (Whale and Singh, 2007). Chroma ( $C^*$ ) refers to the colour saturation and was calculated by the formula of  $(a^{*2} + b^{*2})^{1/2}$ ; while  $h^\circ$  was calculated from the arctangent of  $b^*/a^*$  as demonstrated by McGuire (1992). The CIELAB colour scale below (Figure 3.11) gives a detailed description of colour parameters.

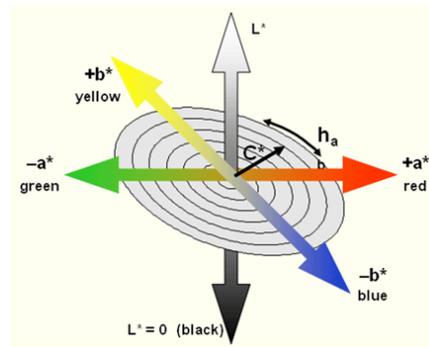


Figure 3.11. CIELAB Colour Scale



Figure 3.12. ColorFlex Spectrophotometer used for colour determination of apple and pear.

**3.7.6. Ascorbic Acid**

Ascorbic acid content was assessed by the method described by Wan Zaliha (2009) with some alterations. Fruit pulp without peel (5 g) was homogenised with an electrical homogenizer (Heidolph DIAX 900, Sydney, Australia) with 25 ml of 6 % metaphosphoric acid solution which contained 0.18 g of EDTA (Ethylenediaminetetraacetic acid) and centrifuged (5810R Eppendorf Centrifuge, Hamburg, Germany) for 20 min at the speed of 5000 g. Then 500  $\mu$ l supernatant was diluted with 3 % metaphosphoric acid (200  $\mu$ l), d. H<sub>2</sub>O (1400  $\mu$ l) and Folin's reagent (200  $\mu$ l, diluted 5 times with d.H<sub>2</sub>O). Following dilution, absorbance of the sample was recorded at 760 nm using ultraviolet-visible spectrophotometry (Model 6405, Dunmow, Essex, UK) (Figure 3.13) and calculation of ascorbic acid was done in mg Kg<sup>-1</sup> using standard curve of L-ascorbic acid.

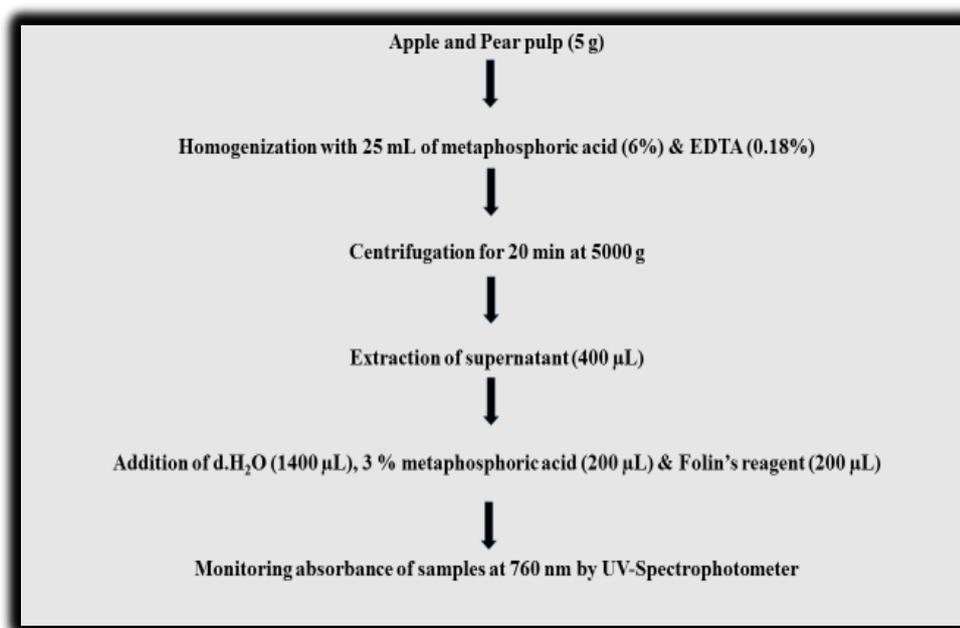


Figure 3.13. Flow diagram for the determination of ascorbic acid concentration in apple and pear pulp.

### 3.7.7. Total Phenolics

Total phenolic content was determined by Folin–Ciocalteu assay as originally described by Singleton and Rossi (1965) and was modified according to Henriquez *et al.* (2010). Fruit pulp (10 g) was homogenized with 90 ml of diluted acetone (70 %) and mixed well in an orbital shaker for 1 hour at 170 g. After mixing, samples were centrifuged at 2500 g for 15 min. Supernatant (0.5 ml) was mixed with d.H<sub>2</sub>O (3 ml) and Folin–Ciocalteu reagent (0.25 ml). Sodium carbonate solution 20 % (0.75 ml) and d.H<sub>2</sub>O (0.95 ml) were added after 1 minute approximately, followed by incubation at 37 °C for half an hour and absorbance was recorded at 765 nm using ultraviolet-visible spectrophotometry (Model 6405, Dunmow, Essex, UK.) (Figure 3.14). Gallic acid (3,4,5-trihydroxybenzoic acid) standard curve was used to calculate total phenolics in apple and pear pulp. The concentration of total phenolics was expressed as mg Kg<sup>-1</sup>.

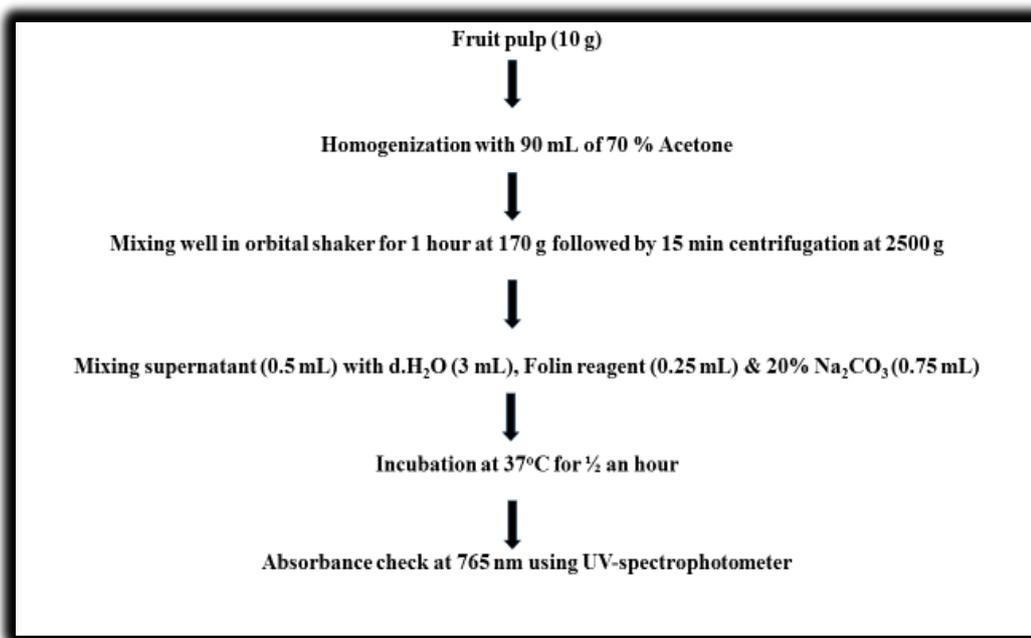


Figure 3.14. Flow diagram for the estimation of total phenolics concentration in apple and pear pulp.

### 3.7.8. Total Antioxidant Capacity

Total antioxidants in apple and pear were quantified by DPPH assay using the protocol of Brand-Williams *et al.* (1995) and Wan Zaliha (2009) with some

modifications. Fruit pulp and peel (15 and 0.5 g respectively) was mashed with an electric homogenizer with 10 ml of extraction buffer comprising of 2 mM sodium fluoride (NaF) solution (dissolved in 200 ml d.H<sub>2</sub>O and 800 ml methanol) and centrifuged at 5000 g for 20 min. Diluted solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) with 1.1 absorbance was mixed (950  $\mu$ l) with different volumes of supernatants which varied with experimental units (15-150  $\mu$ l) and absorbance was checked at 515 nm after 15 min using ultraviolet-visible spectrophotometry (Model 6405, Dunmow, Essex, UK.) (Figure 3.15). Total antioxidant capacity was calculated using standard curve of vitamin E analogue i.e., Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and expressed as mMol Trolox Kg<sup>-1</sup>.

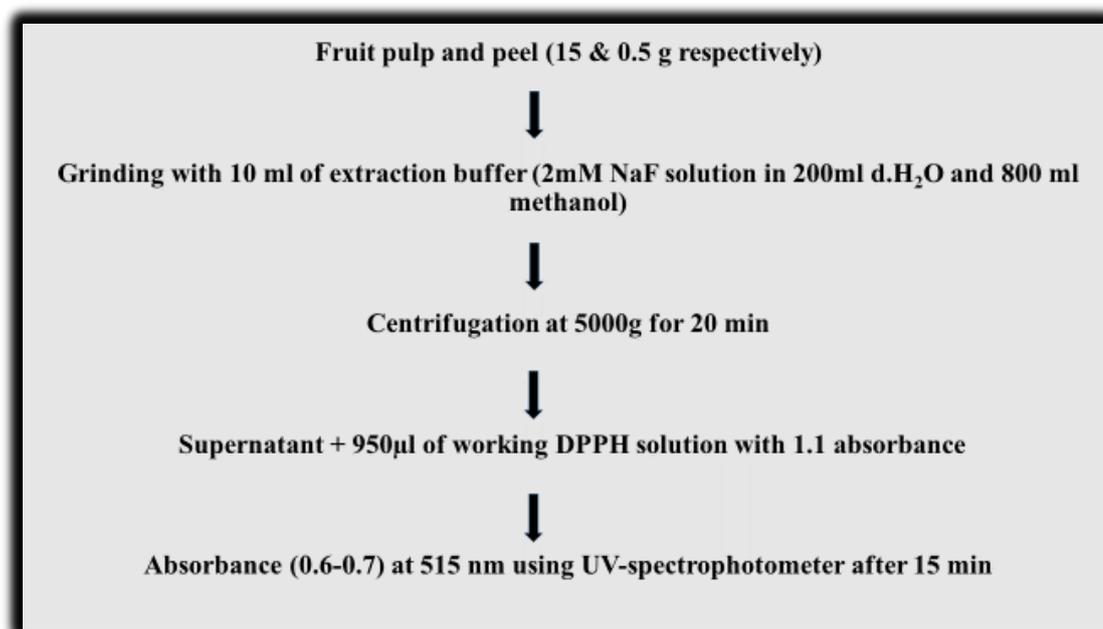


Figure 3.15. Flow chart for the estimation of total antioxidants in peel and pulp of apple and pear by DPPH assay.

### 3.7.9. Individual and Total Organic Acids and Sugars

#### 3.7.9.1. Protocol for sample preparation

Fruit pulp (5 g) was ground with Milli-Q water and the total volume of sample was made up to 50 ml. After homogenization, samples were centrifuged (5810R Eppendorf Centrifuge, Hamburg, Germany) at 10,000 g for 15 min at 4 °C temperature.

Nylon syringe filters (pore size = 0.2  $\mu\text{m}$ ; Thermo Fisher Scientific, Malaga, WA, Australia) were used to filter 1 ml supernatant into HPLC glass vials. High Performance Liquid Chromatography (HPLC; Waters 1525, Milford Corp, MA, USA) was used to determine sugars and organic acids in the filtrate (20  $\mu\text{l}$ ) and were expressed as  $\text{g kg}^{-1}$  pulp of apple and pear fruit (Khan *et al.*, 2016).

### 3.7.9.2. HPLC

Reverse phase liquid chromatography was used in the HPLC system (Waters, 717plus, Milford Corp, MA, USA) to determine the concentrations of individual sugars and organic acids. For the separation of individual sugars and organic acids, Bio-Rad Aminex<sup>®</sup>HPX-87C Fast Carbohydrate Column and Bio-Rad Aminex<sup>®</sup> HPX-87H column (Bio-Rad Laboratories Inc., Hercules, USA) were used respectively. Sulphuric acid solution (0.005M) and degassed water with a flow rate of 0.6  $\text{ml min}^{-1}$  was used for the elution of individual organic acids and sugars respectively. Organic acids were detected by UV-detector (Waters 2487, Milford Corp, MA, USA) at the wavelength of 210 nm while sugars were detected by Refractive Index (RI) detector (Waters 2414, Milford Corp, MA, USA). The individual sugars and organic acids were identified by comparing the retention times of sample's chromatographic peaks with standard's peaks. Data were processed by Breeze<sup>®</sup> software (Figure 3.16 - 3.17 and Table 3.2 - 3.3).

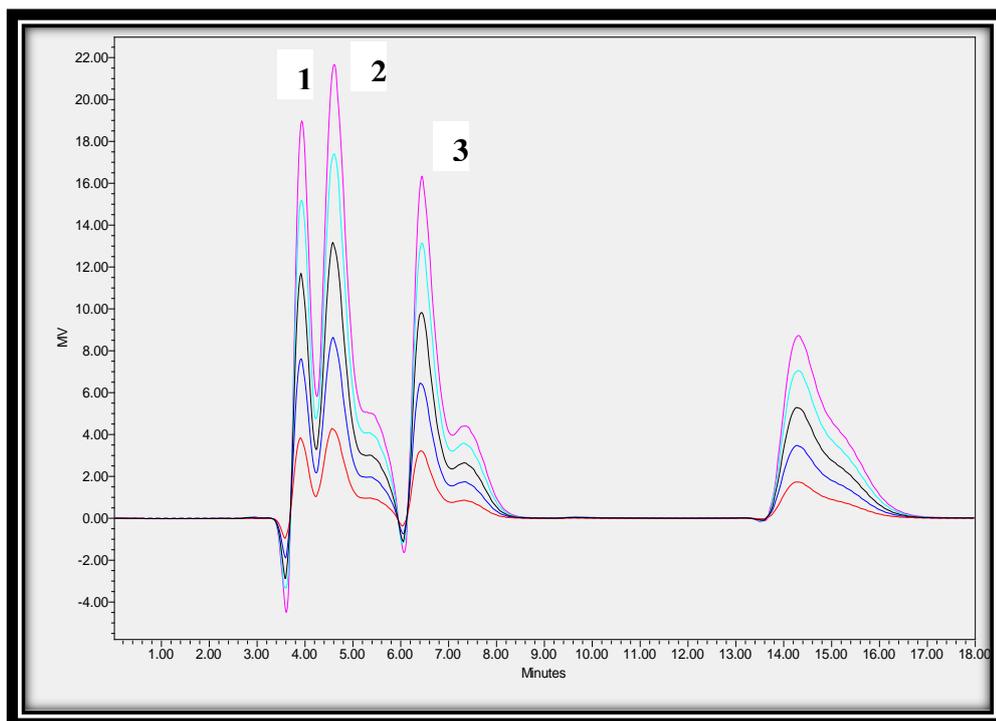


Figure 3.16. HPLC chromatograph of sugar standards used to quantify individual sugars in ‘Fuji’ and ‘Cripps Pink’. Peak 1: Sucrose, Peak 2: Glucose, Peak 3: Fructose.

Table 3.2. Elution order and retention times of sugar standards

Elution order	Standard	Retention time (min)	Detection wavelength (nm)
1	Sucrose	4.053	RI
2	Glucose	4.742	RI
3	Fructose	6.618	RI

RI = Refractive Index

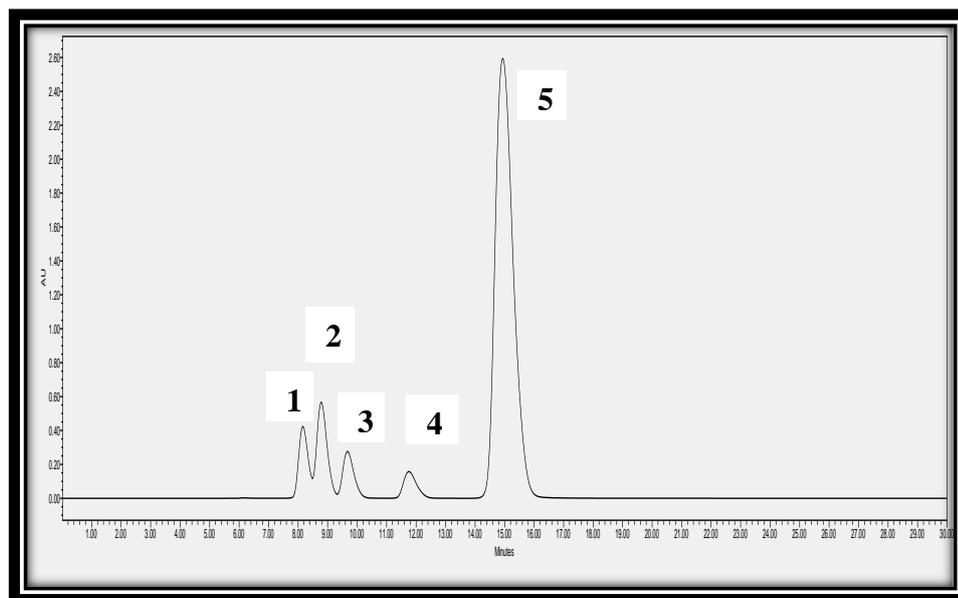


Figure 3.17. HPLC chromatograph of organic acid standards used to quantify individual organic acids in ‘Fuji’ and ‘Cripps Pink’.

Table 3.3. Elution order and retention times of different organic acid standards

Elution order	Standard	Retention time (min)	Detection wavelength (nm)
1	Citric acid	8.054	210
2	Malic acid	9.640	210
3	Succinic acid	11.812	210
4	Fumaric acid	14.227	210
5	Tartaric acid	15.663	210

### 3.8. Experimental Design and Data Analysis

The experiment was laid out following completely randomized design (CRD) with two factors including treatments and storage period, three replications and 20 fruit per replication. Untreated fruit served as a control. The experimental data were statistically analysed using comprehensive statistical package GenStat 14.1 (VSN International Ltd. Hemel Hempstead, UK) by Analysis of Variance (ANOVA) technique. Differences among treatment means were compared by least significant difference test (LSD) at the probability level of 5 %.

## CHAPTER 4

### Efficacy of Ethylene Antagonists in Extending Cold Storage Life and Maintaining Fruit Quality of 'Fuji' and 'Cripps Pink' Apple

#### Abstract

Apple is a climacteric fruit and its quality and storage life are adversely affected by climacteric ethylene production during storage and ripening. The use of ethylene antagonists is a practical approach to inhibit ethylene action in order to protect fruit from the deleterious effects of ethylene. The present investigations were conducted to assess the effects of different ethylene antagonists *viz.*, 1-methylcyclopropene (1-MCP), 1-hexylcyclopropene (1-HCP), (*S*)-(-)-limonene and *trans*-cinnamaldehyde (TCA) on ethylene production, respiration rate, storage life and various fruit quality parameters of 'Fuji' and 'Cripps Pink' apple cultivars in cold storage conditions. The apple fruit were fumigated with 1  $\mu$ M solution of the above-mentioned ethylene antagonists except 1-MCP which was used at the rate of 740 ppb in sealed plastic containers for 24 hours. Untreated fruit were kept as a control. Following the fumigation treatments, the fruit were stored at  $0.5 \pm 0.5$  °C temperature and  $85 \pm 5$  % R.H. for a period of 28, 75 and 120 days. Following each cold storage period, ethylene production and respiration rate were determined daily until post-climacteric phase. Whilst, all the other fruit quality parameters were estimated after 10 days of simulated shelf condition at ambient temperature. Maximum suppression in climacteric ethylene production was recorded with 1-MCP fumigation treatment followed by (*S*)-(-)-limonene and TCA after 28 days cold storage of 'Fuji' fruit with delayed climacteric peak in both apple cultivars. All ethylene antagonists were parallel to each other in suppressing respiratory rate with more pronounced effect of 1-MCP, however, maximum delay in respiratory climacteric peak was found with (*S*)-(-)-limonene in 'Cripps Pink' apple fruit. Effect of ethylene antagonist's fumigation treatments on TA, SSC and SSC/TA ratio was not conspicuous without any major changes in these parameters; while loss of firmness was inhibited to varying levels commendably by all tested ethylene antagonists depending upon storage intervals. Ethylene antagonists were found to be effective in maintaining the quality attributes of both cultivars including ascorbic acid, total phenolics, antioxidants and sugars as compared to the control; whereas their effects on levels of organic acids were inconsistent. 1-MCP

fumigation was highly effective to maintain all quality parameters of both cultivars, nonetheless, 1-HCP, (S)-(-)-limonene and TCA also had a positive impact on various quality attributes depending upon cultivar and cold storage period. Hence, it is suggested that 1-HCP, (S)-(-)-limonene and TCA have potential for exploitation by the apple industry as an alternative to 1-MCP for postharvest quality and storability management of apple cultivars.

### 4.1. Introduction

Apple is one of the five most consumed fruits worldwide and constitutes an important part of the human diet being a rich source of antioxidants and phenolic compounds, reducing the risk of cardiovascular disease and its causal factors (Hyson, 2011). Being a perishable fruit, apple demands effective measures to extend storage life while simultaneously maintaining its quality (Morales *et al.*, 2010). Ethylene is one of the phytohormones that plays an important role in plant growth, development and senescence (Bapat *et al.*, 2010). From the postharvest perspective, ethylene is a chief regulator of fruit ripening, quality and storability by stimulating a complicated signalling cascade (Ju and Chang, 2012). Presence of ethylene during postharvest handling and storage leads to major physiological and compositional changes in fruit, ultimately deteriorating its quality and making it unacceptable for the end users (Ansari and Tuteja, 2015). Autocatalytic ethylene production in apples due to its climacteric nature results in aging, fruit softening, certain disorders like scald, discoloration, fruit decay and bitter flavour resulting in a net decrease in storage life (Yang *et al.*, 2013). Generally, these damaging effects of ethylene on fresh produce account for 10-80 % losses at various steps which provoke the need for productive postharvest strategies to address the future threats of food security (Gogo *et al.*, 2017).

Postharvest technology provides some new avenues to minimize fresh produce losses by inhibition of ethylene biosynthesis or action with genetic or chemical approaches (Ansari and Tuteja, 2015). Inhibition of ethylene biosynthesis is achieved by suppressing the activities of ACS (1-aminocyclopropane-1-carboxylate synthase) and ACO (1-aminocyclopropane 1-carboxylate oxidase) enzymes using AVG (aminoethoxyvinylglycine), MVG (methoxyvinylglycine) and AOA (aminooxyacetic acid). This approach is not always successful as it does not protect fruits from exogenous sources of ethylene (Martinez-Romero *et al.*, 2007). Hence, inhibition of

ethylene action with ethylene antagonists propounds an effective tool to combat postharvest losses by providing protection from endogenous as well as exogenous sources of ethylene (Sisler *et al.*, 2006). Ethylene antagonists block receptor sites where binding of ethylene triggers complicated signalling pathways, ultimately leading to a burst in ethylene production and concomitant loss of fruit quality (Keller *et al.*, 2013). In apples, ethylene antagonists have been reported to slow down ethylene dependent ripening, softening, fruit breakdown, scald formation and loss of flavour (DeEll *et al.*, 2007; Elfving *et al.*, 2007). Cyclopropenes are nontoxic ethylene antagonists for the agro-food industry with particular focus on 1-MCP which has been exploited significantly to manage postharvest challenges in apple with productive outcomes (Xu *et al.*, 2016).

Although, 1-MCP (SmartFresh™) is a significant component of the apple industry due to its efficacy in delaying fruit ripening, softening and extending storage life, it has some critical drawbacks like flesh browning in certain apple cultivars (James *et al.*, 2010; Watkins and Nock, 2012) and stem and russet browning of 'Cox's Orange Pippin' cultivar (McCormick and Streif, 2007). The situation is getting worse with some cases of 'lack of flavour' and excessive hard texture in 1-MCP treated apples (Watkins and Nock, 2004; Marin *et al.*, 2009). Moreover, effect of 1-MCP on postharvest quality of apple and other fruits is highly genotypic dependent demanding strict optimization of application variables (Watkins and Nock, 2004). Higher cost of 1-MCP further limits its commercial application particularly in developing countries (Wang *et al.*, 2006). All these issues are of concern to the apple industry demanding some novel alternatives to overcome the shortcomings of 1-MCP and other ethylene antagonists.

Use of cyclopropene derivatives as ethylene antagonists is a recent innovation as they inhibit the deleterious effects of ethylene like 1-MCP (Xu *et al.*, 2016). 1-Hexylcyclopropene (1-HCP) is a structural analogue of 1-MCP with a longer carbon chain at 1-position and it has been used in practical attempts using kalanchoe and tomato with fruitful results (Serek *et al.*, 2007; Khan *et al.*, 2016). Apart from synthetic ethylene antagonists, there are a few natural growth regulators like (S)-(-)-limonene from essential oils of some aromatic plants which have been used in avocado (Regnier *et al.*, 2010), mango (Regnier *et al.*, 2008), pepper, spinach (Lu *et al.*, 2012 and 2013b), apricot (Zhao and Yang, 2014) and nectarine (Zhao *et al.*, 2015) to maintain fruit

quality and as a defensive agent against postharvest diseases. In addition, *trans*-cinnamaldehyde (TCA), a naturally occurring flavonoid from cinnamon known for its anti-microbial activity effectively reduced postharvest quality damages in tomato (Tzortzakis, 2009) sweet pepper (Xing *et al.*, 2011), rambutan (Sivakumar *et al.*, 2002) and melon (Carvalho *et al.*, 2016). 1-HCP, TCA and (*S*)-(-)-limonene have also been found to reduce the ethylene induced abscission in waxflower by Abdalghani (2017). However, no research work has been reported on apple using these ethylene antagonists.

Furthermore, the efficacy of cold storage for apples is proven by studies for delayed ethylene production, hindered respiratory climacteric, reduced loss of acidity and increased levels of carotenoids (Ticha *et al.*, 2008). However, there is significant accumulation of ethylene in cold rooms from varying sources like normal emission from stored produce, fungal metabolism or pollutants in the atmosphere which cannot be removed entirely by conventional methods (Janssen *et al.*, 2014). Combination of cold storage with ethylene antagonists can be more effective in extending storage life and maintaining fruit quality of apple as it would provide a safe storage environment by inhibiting ethylene action (Hoang *et al.*, 2011). Moreover, some studies suggest that low temperatures during cold storage act as an inducer of ethylene biosynthesis in different apple cultivars after shifting fruit to ambient conditions by increasing the ACC (1-aminocyclopropane-1-carboxylic acid) concentration (Larrigaudiere *et al.*, 1997). Ethylene antagonists can therefore serve as a protective treatment for cold stored apples even after removal from storage chambers.

As a prelude, 1-HCP reduced ethylene production and delayed ripening in tomato fruit which is a climacteric fruit like apple, while TCA and (*S*)-(-)-limonene inhibited ethylene dependent abscission in waxflower. Hence, it was hypothesized that 1-HCP, TCA and (*S*)-(-)-limonene can inhibit ethylene deteriorating effects in apple fruit. Therefore, the effect of 1-MCP, 1-HCP, TCA and (*S*)-(-)-limonene fumigation on ethylene production, storage life and maintaining fruit quality of 'Fuji' and 'Cripps Pink' apple was investigated under cold storage conditions. These natural compounds have potential to be used for the long-term storage of apple fruit and can help greatly to reduce the associated environmental footprints.

## 4.2. Materials and Methods

### 4.2.1. Experiments

Two independent experiments were conducted to test the efficacy of new ethylene antagonists on ethylene production, respiration rate, extending cold storage life and maintaining quality of 'Fuji' and 'Cripps Pink' apple cultivars. Experiment details are given below.

#### 4.2.1.1. Experiment 1: Effect of ethylene antagonists in extending cold storage life and maintaining fruit quality of apple cv. 'Fuji'.

In this experiment, four different ethylene antagonists i.e., 1-MCP (740 ppb), 1-HCP (1  $\mu$ M), (*S*)-(-)-limonene (1  $\mu$ M) and TCA (1  $\mu$ M) were used to fumigate 'Fuji' apple fruit. Experiment was laid out as two factor factorials (ethylene antagonists  $\times$  cold storage periods) completely randomized design (CRD) with five treatments. Each treatment was replicated 3 times with 20 fruit in each replication.

After fumigation with ethylene antagonists, both treated and untreated (control) 'Fuji' fruit were stored in cold rooms at  $0.5\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$  temperature and  $85 \pm 5\%$  relative humidity (R.H.) for a period of 28, 75 and 120 days. After the completion of cold storage periods, 'Fuji' fruit were shifted to simulated shelf conditions ( $21 \pm 1\text{ }^{\circ}\text{C}$ ). Ethylene production and respiration rate were monitored daily until post-climacteric period in the fruit which were stored for 28, 75 and 120 days. Following 75 and 120 days of cold storage, fruit firmness, colour, SSC, TA, SSC/TA ratio, ascorbic acid, total phenolics, total antioxidants, individual and total sugars, as well as organic acids were determined on 10<sup>th</sup> day of simulated shelf conditions. Temperature and R.H. was monitored during cold storage using Tiny Tags (Gemini Data Loggers, West Sussex, UK) run by Tinytag Explorer Software.

#### 4.2.1.2. Experiment 2: Effect of ethylene antagonists in extending cold storage life and maintaining fruit quality of apple cv. 'Cripps Pink'.

This experiment was conducted to evaluate the efficacy of 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA on cold storage life and fruit quality of 'Cripps Pink' apple fruit. 1-HCP, (*S*)-(-)-limonene and TCA were used at the strength of 1  $\mu$ M while commercial formulation of 1-MCP was used with the concentration of 740 ppb. Experiment was

designed as two-factor CRD (ethylene antagonists  $\times$  cold storage periods) with five treatments and three replications in each treatment. Number of fruit in each replication was twenty. 'Cripps Pink' fruit were stored in cold rooms after fumigation treatment with ethylene antagonists at  $0.5\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$  temperature and  $85 \pm 5\%$  relative humidity (R.H.) for a period of 28, 75 and 120 days. Data were recorded for rate of ethylene production, respiration rate and various fruit quality parameters as detailed in experiment 1 for 'Fuji' apple.

### 4.2.2. Plant Material and Experimental Site

Apple fruit (*Malus  $\times$  domestica* Borkh.) cv. 'Fuji' and 'Cripps Pink' were obtained from a commercial grower in Manjimup, located at latitude  $34^{\circ}14'$  South and longitude  $116^{\circ} 8'$  East, Western Australia (WA). Mature fruit, free from diseases, nutritional deficiencies and bruises were used in the experiments. Fruit were transported in a refrigerated truck following harvest to the Horticulture Research Laboratory, School of Molecular and Life Sciences, Bentley Campus, Curtin University, Perth, WA and used for various experiments.

### 4.2.3. Fumigation with Ethylene Antagonists

After grading, uniform sized apple fruit were selected for treatment with ethylene antagonists *viz.*, 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA which were bought from different companies. Fruit were fumigated with  $1\text{ }\mu\text{M}$  solution of the above-mentioned ethylene antagonists in an airtight plastic container for 24 hours. Solutions of ethylene antagonists (5 ml) were placed inside the container in a small Petri dish with a battery-operated fan for equal dissemination of chemical fumes. For 1-MCP fumigation treatment, tablets of commercial formulation were used having concentration of 740 ppb and were dipped in buffer solution before placing inside sealed containers. Soda lime (25 g) in a Petri dish was also placed inside each container to absorb excessive  $\text{CO}_2$ . Untreated fruit served as a control.

### 4.2.4. Ethylene Production and Respiration Rate

Ethylene production rate from apple fruit was determined using a laser based system (ETD-300; Sensor Sense B.V., Nijmegen, The Netherlands) as elaborated previously by Cristescu *et al.* (2013) and expressed as  $\text{pmol kg}^{-1} \text{ s}^{-1}$ . To determine respiration rate, the fruit were kept in sealed glass jars of 1 L volume fitted with a

septum (SubaSeal®, Sigma-Aldrich Co., St. Louis, USA) at room temperature. After 1-2 hours incubation of fruit, headspace gas samples (1 ml) were used to estimate CO<sub>2</sub> concentration by using an infrared gas analyser (Servomex Series 1400, Sussex, UK). The respiration rate was expressed as  $\mu\text{mol kg}^{-1} \text{s}^{-1}$ .

### 4.2.5. Fruit Firmness

Firmness was estimated as resistance to penetration on two opposite equatorial sides of fruit using a texture analyser (TA Plus, Lloyds Instruments, Hampshire, UK) fitted with 11.1 mm diameter plunger and run by Nexygen™ Plus Material Testing Software. Fruit firmness was expressed in Newtons (N).

### 4.2.6. TA, SSC and SSC/TA Ratio

Juice was extracted from 20 fruit per replication to estimate TA and SSC. The juice (10 ml) was diluted with 20 ml distilled water (d.H<sub>2</sub>O). Following the dilution, 5ml aliquot was titrated against 0.1N NaOH (sodium hydroxide). Phenolphthalein (3,3-bis(4-hydroxyphenyl)-2-benzofuran-1(3*H*)-one) was used to indicate the end of titration by turning juice colour to pink. Acidity was expressed as malic acid equivalents (%). A battery-operated refractometer (ATAGO™ Digital Palette Refractometer, PR-101, Tokyo, Japan) was used to determine SSC from juice samples and expressed as a percentage. SSC/TA ratio was calculated by dividing the values of SSC by the corresponding TA values.

### 4.2.7. Individual and Total Sugars and Organic Acids

Fruit pulp (5 g) was ground with Milli-Q water and the total volume of sample was made up to 50 ml. Following homogenization, the samples were centrifuged (5810R Eppendorf Centrifuge, Hamburg, Germany) at 10,000 g for 15 min at 4 °C temperature. Nylon syringe filters (pore size = 0.2  $\mu\text{m}$ ; Thermo Fisher Scientific, Malaga, WA, Australia) were used to filter one ml of supernatant into HPLC glass vials. High performance liquid chromatography (HPLC; Waters, 717 plus, Milford Corp, MA, USA) was used to determine sugars and organic acids in the filtrate (20  $\mu\text{l}$ ) using refractive index detector and UV-absorbance detector respectively. The detailed conditions of HPLC analytical method have been described earlier in Chapter 3, Section 3.7.9. The concentrations of individual and total organic acids and sugars were expressed as  $\text{g kg}^{-1}$  pulp.

#### **4.2.8. Ascorbic Acid**

The concentration of ascorbic acid was estimated by the method as described previously by Wan Zaliha (2009) with some modifications. Apple pulp without peel (5 g) was homogenised with an electrical homogenizer (Heidolph DIAX 900, Sydney, Australia) using 25 ml of 6 % metaphosphoric acid solution containing 0.18 g of EDTA (ethylenediaminetetraacetic acid) and centrifuged (5810R Eppendorf Centrifuge, Hamburg, Germany) at 5000 g for 20 min. The supernatant (500 µl) was diluted with 3 % metaphosphoric acid (200 µl), d.H<sub>2</sub>O (1400 µl) and Folin's reagent (200 µl, diluted 5 times with d.H<sub>2</sub>O). Absorbance of the sample was recorded at 760 nm using an ultraviolet-visible spectrophotometer (Model 6405, Dunmow, Essex, UK.). The concentration of ascorbic acid was calculated using standard curve of L-ascorbic acid and expressed as mg Kg<sup>-1</sup>.

#### **4.2.9. Total Phenolics**

The concentration of total phenolic in pulp was determined using Folin–Ciocalteu assay as originally detailed by Singleton and Rossi (1965) and was modified according to Henriquez *et al.* (2010). Apple pulp (10 g) was homogenized with 90 ml of diluted acetone (70 %, v/v) and mixed well in an orbital shaker (Ratek, OM7, Rowe Scientific, Perth, WA) for 1 hour at 170 rpm. After mixing, the sample was centrifuged (5810R Eppendorf Centrifuge, Hamburg, Germany) at 2500 g for 15 min. Supernatant (0.5 ml) was mixed with d.H<sub>2</sub>O (3 ml) and Folin–Ciocalteu reagent (0.25 ml). Sodium carbonate solution 20 % (0.75 ml) and d.H<sub>2</sub>O (0.95 ml) were added after one minute approximately, followed by incubation at 37 °C for half an hour and absorbance was recorded at 765 nm using ultraviolet-visible spectrophotometer (Model 6405, Dunmow, Essex, UK.). The concentration of total phenolics was calculated using gallic acid (3,4,5-trihydroxybenzoic acid) standard curve and expressed as mg Kg<sup>-1</sup> gallic acid equivalents.

#### **4.2.10. Total Antioxidants**

The concentrations of total antioxidants in the fruit pulp were quantified by employing DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described by Brand-William *et al.* (1995) with some modifications (Wan Zaliha, 2009). Apple pulp along with peel (15 and 0.5 g respectively) was mashed with an electric homogenizer with

10 ml of extraction buffer comprising of 2 mM sodium fluoride (NaF) solution (NaF dissolved in 200 ml d.H<sub>2</sub>O and 800 ml methanol) and centrifuged (5810R Eppendorf Centrifuge, Hamburg, Germany) at 5000 g for 20 min. Diluted solution of DPPH (950 µl) with 1.1 absorbance was mixed with different volumes of supernatants (15-150 µl). The absorbance was recorded at 515 nm after 15 min using ultraviolet-visible spectrophotometer (Model 6405, Dunmow, Essex, UK.). The concentration of total antioxidants was calculated using standard curve of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and expressed as mMol Trolox Kg<sup>-1</sup>.

### 4.2.11. Statistical Analysis of Data

The data were statistically analysed using comprehensive statistical package GenStat 14.1 by analysis of variance (ANOVA). Differences among treatment means were compared by least significant difference test (LSD) at the probability level of 5 %.

## 4.3. Results

### 4.3.1. Ethylene Production

Fumigation treatments of 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA significantly decreased mean climacteric ethylene production in 'Fuji' and 'Cripps Pink' apple fruit which were kept at ambient temperature following 28, 75 and 120 days cold storage (CS). In both the cultivars, the cold storage period also significantly influenced mean climacteric ethylene production. The interactions between different fumigation treatments and cold storage periods were found to be significant for ethylene production in both the cultivars (Table 4.1 and 4.2).

The climacteric ethylene peak in 'Fuji' apple was significantly delayed by 4 and 3 days when treated with TCA and (*S*)-(-)-limonene respectively after 28 days CS as compared to the control. Whilst, following 75 days CS, 1-HCP treated fruit showed significantly delayed climacteric ethylene peak (7.6 days) as compared to control and all other treatments. In 120 days stored 'Fuji' fruit, climacteric peaks were significantly delayed by 6.3 and 6 days with 1-MCP and TCA respectively as compared to the control (Table 4.1). When averaged over different cold storage periods, the fumigation of 1-MCP, 1-HCP and TCA significantly delayed the climacteric ethylene production

peak (5, 5 and 5.11 days respectively) as compared to the control and (*S*)-(-)-limonene fumigated fruit (2.45 days) in 'Fuji' apple (Table 4.1).

In 'Cripps Pink', there was a delay of 4, 3.3 and 2.3 days in climacteric peak when fruit were treated with 1-MCP, (*S*)-(-)-limonene and TCA treatments respectively following 28 days cold storage as compared to the control. All the ethylene antagonists have significantly delayed climacteric ethylene peak (2.6 to 3.3 days) in 75 days cold stored fruit as compared to the control. 'Cripps Pink' apple fruit fumigated with 1-MCP and TCA showed a considerable delay in climacteric peak (3.7 and 2.7 days) in 120 days cold stored fruit as compared to control and all other treatments (Table 4.2). Treatment means for 'Cripps Pink' over different cold storage periods indicated that apple fruit fumigated with 1-MCP, (*S*)-(-)-limonene, and TCA had a delayed onset of climacteric peak (3.5, 2.5 and 2.7 days respectively) as compared to the control (Table 4.2). Means of different storage periods showed that number of days to the onset of climacteric peak reduced with an extension of cold storage time for both cultivars (Table 4.1 and 4.2).

The rate of climacteric ethylene peak was significantly lowest in 1-MCP, (*S*)-(-)-limonene, and TCA treated 'Fuji' fruit after 28 days CS i.e. 126.4, 142.5 and 184.1 pmol kg<sup>-1</sup> s<sup>-1</sup> as compared to the control and 1-HCP. However, after 75 and 120 days cold storage of 'Fuji', concentration of ethylene was suppressed remarkably only with 1-MCP fumigation treatment (101.3 and 125.2 pmol kg<sup>-1</sup> s<sup>-1</sup> respectively). When averaged over different storage periods, mean climacteric ethylene concentration was significantly reduced (117.6 pmol kg<sup>-1</sup> s<sup>-1</sup>) in 1-MCP treated 'Fuji' fruit, followed by TCA treated fruit (708.1 pmol kg<sup>-1</sup> s<sup>-1</sup>) as compared to all other treatments and control (1384.1 pmol kg<sup>-1</sup> s<sup>-1</sup>) (Table 4.1).

Similarly to 'Fuji', rate of climacteric ethylene peak in 'Cripps Pink' was significantly reduced with 1-MCP fumigation after 28, 75 and 120 days of CS (89, 98 and 110 pmol kg<sup>-1</sup> s<sup>-1</sup> respectively) as compared to control and all other treatments (Table 4.2). Mean concentration of ethylene for different fumigation treatments over cold storage periods in 'Cripps Pink' showed significantly less value (99.0 pmol kg<sup>-1</sup> s<sup>-1</sup>) with 1-MCP fumigation as compared to all other treatments and control. Whilst, 1-HCP, (*S*)-(-)-limonene, and TCA fumigation treatments also significantly reduced mean climacteric ethylene production as compared to the control but the difference

among these treatments was not significant (Table 4.2). The highest mean climacteric ethylene concentration in 'Fuji' and 'Cripps Pink' (1180.4 and 1529 pmol kg<sup>-1</sup> s<sup>-1</sup> respectively) was observed in 120 days cold stored fruit as compared to those stored for 28 and 75 days (Table 4.1 and 4.2).

Table 4.1. Rate of climacteric ethylene peak and number of days to the onset of ethylene peak in apple cv. 'Fuji' as affected by ethylene antagonists and cold storage periods.

Ethylene antagonists	Climacteric Peak			Mean (T)	Peak Onset			Mean (T)
	Ethylene (pmol kg <sup>-1</sup> s <sup>-1</sup> )				(Days)			
	28 d	75 d	120 d		28 d	75 d	120 d	
Control	1086 ± 20.7cd	1233.9 ± 27.1d	1832.4 ± 79.8e	1384.1d	14.0 ± 0.00f	5.7 ± 0.27b	2.0 ± 0.00a	7.22a
1-MCP	126.4 ± 24.4a	101.3 ± 0.6a	125.2 ± 1.3a	117.6a	14.3 ± 0.49f	11.0 ± 0.62e	8.3 ± 0.14d	11.22c
1-HCP	859.8 ± 83.5bc	862.4 ± 25.6bc	1150.8 ± 19.1d	957.7c	14.3 ± 0.68f	13.3 ± 0.14f	6.0 ± 0.00bc	11.22c
(S)-(-)-Lim	142.5 ± 6.1a	1105.6 ± 6.9cd	1657.3 ± 64.1e	968.5c	17.0 ± 0.00g	9.0 ± 0.00de	3.0 ± 0.00a	9.67b
TCA	184.1 ± 10.7a	803.9 ± 25.0b	1136.2 ± 8.5cd	708.1b	18.0g ± 0.00	11.0 ± 0.00e	8.0 ± 0.00cd	12.33c
Mean (SP)	479.8a	821.4b	1180.4c		15.5c	10.0b	5.47a	
LSD ( $P \leq 0.05$ )	T = 147.1	SP = 39.3	T × SP = 87.9		T = 1.18	SP = 0.91	T × SP = 2.04	

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = treatment, SP = storage period (days), T × SP = Interaction of treatments and storage periods. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 4.2. Rate of climacteric ethylene peak and number of days to the onset of ethylene peak in apple cv. 'Cripps Pink' as affected by ethylene antagonists and cold storage periods.

Ethylene antagonists	Climacteric Peak			Mean (T)	Peak Onset			Mean (T)
	Ethylene (pmol kg <sup>-1</sup> s <sup>-1</sup> )				(Days)			
	28 d	75 d	120 d		28 d	75 d	120 d	
Control	1585 ± 103.5defg	1586 ± 62.2defg	2154 ± 31.7h	1775c	6.7 ± 0.14b	4.7 ± 0.14a	4.0 ± 0.00a	5.1a
1-MCP	89 ± 3.4a	98 ± 0.7a	110 ± 1.3a	99a	10.7 ± 0.27d	7.3 ± 0.59bc	7.7 ± 0.27bc	8.6c
1-HCP	1035 ± 17.6b	1398 ± 60.1cde	1788 ± 49.2fg	1407b	7.0 ± 0.00bc	7.7 ± 0.14bc	4.0 ± 0.00a	6.2b
(S)-(-)-Lim	993 ± 14.2b	1447 ± 40.2cdef	1864 ± 93.8gh	1435b	10.0 ± 0.00d	8.0 ± 0.00bc	4.7 ± 0.14a	7.6c
TCA	1156 ± 68.9bc	1305 ± 46.7bcd	1727 ± 53.0efg	1396b	9.0 ± 0.00cd	7.7 ± 0.36bc	6.7 ± 0.54b	7.8c
Mean (SP)	972a	1167b	1529c		8.7c	7.1b	5.4a	
LSD ( $P \leq 0.05$ )	T = 192.7	SP = 149.3	T × SP = 333.8		T = 1.02	SP = 0.79	T × SP = 1.77	

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = treatment, SP = storage period (days), T × SP = Interaction of treatments and storage periods. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

### 4.3.2. Respiration Rate

Fumigation of 'Fuji' and 'Cripps Pink' with different ethylene antagonists *viz.*, 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA significantly reduced the respiration rate and delayed the onset of respiratory climacteric in both apple cultivars after cold storage at ambient temperature. The interaction between different cold storage periods (28, 75 and 120 days) and fumigation treatments was significant for respiratory parameters in both cultivars (Table 4.3 and 4.4).

Onset of respiratory climacteric peak in 'Fuji' after 28 days CS was delayed by 3 and 2 days with 1-HCP and TCA treatments respectively in comparison to control. After 75 days CS, respiratory peak was delayed by 2 days with (*S*)-(-)-limonene; while in 120 days cold stored 'Fuji', the delay was 5 and 4.3 days with (*S*)-(-)-limonene and TCA treatments as compared to control. When averaged over different cold storage periods, mean respiratory peak days are non-significant for 'Fuji'. 'Fuji' fruit cold stored for 28 days, exhibited significantly delayed onset of mean respiratory peak (11.7 days) compared to those stored for 75 and 120 days (6.9 and 5.9 days respectively) (Table 4.3).

In 'Cripps Pink', (*S*)-(-)-limonene and TCA treatments led to a delay in respiratory climacteric peak by 4.7 and 3.3 days as compared to the control after 28 and 75 days CS respectively. (*S*)-(-)-limonene treatment also significantly delayed the onset of mean respiratory peak by 2.4 days as compared to the control and all other treatments except 1-MCP and TCA (Table 4). 'Cripps Pink' fruit cold stored for 28 and 75 days showed significantly delayed onset of mean respiratory peak (6.7 and 6.9 days) compared to those stored for 120 days (5.7 days) (Table 4.4).

All the treatments significantly reduced the rate of respiratory peak in 28 days cold stored 'Fuji' fruit as compared to the control. In 1-MCP treated fruit, significantly reduced respiratory peak ( $0.23 \mu\text{mol kg}^{-1} \text{s}^{-1}$ ) was recorded after 120 days CS as compared to the control and all other treatments. Likewise, 1-MCP treatment resulted in significantly reduced mean respiration rate ( $0.29 \mu\text{mol kg}^{-1} \text{s}^{-1}$ ) in 'Fuji' as compared to control and all other treatments except (*S*)-(-)-limonene (Table 4.3).

All the treatments suppressed the respiration peak rates in 'Cripps Pink' as compared to the control depending upon cold storage periods. 1-MCP fumigation

resulted in lowest mean rate of respiration ( $0.26 \mu\text{mol kg}^{-1} \text{s}^{-1}$ ) as compared to the control and all other treatments (Table 4.4). Mean respiration rates varied significantly between cold storage periods in both cultivars (Table 4.3 and 4.4).

Table 4.3. Rate of respiratory peak and number of days to the onset of respiratory climacteric peak in apple cv. 'Fuji' as affected by ethylene antagonists and cold storage periods.

Ethylene antagonists	Climacteric Peak			Mean (T)	Peak Onset			Mean (T)
	Respiration ( $\mu\text{mol kg}^{-1} \text{ s}^{-1}$ )				(Days)			
	28 d	75 d	120 d		28 d	75 d	120 d	
Control	0.85 $\pm$ 0.08c	0.52 $\pm$ 0.02b	0.53 $\pm$ 0.03b	0.63c	11.3 $\pm$ 0.36fg	8.7 $\pm$ 0.72def	3.0 $\pm$ 0.00a	7.7
1-MCP	0.31 $\pm$ 0.01ab	0.35 $\pm$ 0.02ab	0.23 $\pm$ 0.01a	0.29a	11.7 $\pm$ 0.54gh	6.7 $\pm$ 0.14bcd	4.7 $\pm$ 0.14ab	7.7
1-HCP	0.52 $\pm$ 0.03b	0.53 $\pm$ 0.05b	0.37 $\pm$ 0.01ab	0.47b	14.3 $\pm$ 0.27h	3.3 $\pm$ 0.14a	6.3 $\pm$ 0.49bcd	8.0
(S)-(-)-Lim	0.38 $\pm$ 0.01ab	0.48 $\pm$ 0.02ab	0.34 $\pm$ 0.01ab	0.40ab	8.0 $\pm$ 0.41cde	10.7 $\pm$ 0.36efg	8.0 $\pm$ 0.00cde	8.8
TCA	0.46 $\pm$ 0.05ab	0.51 $\pm$ 0.03b	0.37 $\pm$ 0.00ab	0.45b	13.3 $\pm$ 0.36gh	5.3 $\pm$ 0.54abc	7.3 $\pm$ 0.27bcd	8.7
Mean (SP)	0.50b	0.48b	0.37a		11.7b	6.9a	5.9a	
LSD ( $P \leq 0.05$ )	T = 0.14	SP = 0.11	T $\times$ SP = 0.24		T = NS	SP = 1.15	T $\times$ SP = 2.6	

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1 $\mu$ M), TCA = *trans*-cinnamaldehyde (1 $\mu$ M), T = treatment, SP = storage period (days), T  $\times$  SP = Interaction of treatments and storage periods.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 4.4. Rate of respiratory peak and number of days to the onset of respiratory climacteric peak in apple cv. 'Cripps Pink' as affected by ethylene antagonists and cold storage periods.

Ethylene antagonists	Climacteric Peak			Mean (T)	Peak Onset			Mean (T)
	Respiration ( $\mu\text{mol kg}^{-1} \text{s}^{-1}$ )				(Days)			
	28 d	75 d	120 d		28 d	75 d	120 d	
Control	0.45±0.03bc	0.58±0.02cd	0.64±0.04d	0.55c	4.3±0.4a	4.7±0.1a	5.7±0.1abcd	4.9a
1-MCP	0.23±0.01a	0.30±0.02ab	0.26±0.02a	0.26a	7.3±0.1bcdefg	7.7±0.5cdefg	6.0±0.0abcde	7.0bc
1-HCP	0.35±0.00ab	0.43±0.01bc	0.58±0.02cd	0.45b	5.7±0.4abc	7.0±0.2bcdef	5.3±0.1ab	6.0b
(S)-(-)-Lim	0.32±0.01ab	0.42±0.00bc	0.38±0.01ab	0.37b	9.0±0.0g	7.3±0.1bcdefg	5.7±0.5abc	7.3c
TCA	0.32±0.01ab	0.46±0.03bc	0.43±0.02bc	0.40b	7.3± 0.1bcdefg	8.0± 0.2fg	5.7±0.1abc	7.0bc
Mean (SP)	0.33a	0.44b	0.46c		6.7b	6.9b	5.7a	
LSD ( $P \leq 0.05$ )	T = 0.08	SP = 0.06	T×SP = 0.14		T = 1.0	SP = 0.8	T×SP = 1.7	

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu\text{M}$ ), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu\text{M}$ ), TCA = *trans*-cinnamaldehyde (1  $\mu\text{M}$ ), T = treatment, SP = storage period (days), T × SP = Interaction of treatments and storage periods.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

### **4.3.3. Firmness**

Mean fruit firmness of 1-MCP fumigated 'Fuji' was significantly the highest (76.9 N) as compared to the control and all other fumigation treatments (Table 4.5). Meanwhile, the fruit fumigated with TCA, (*S*)-(-)-limonene and 1-HCP exhibited significantly higher fruit firmness as compared to the control. Mean firmness was significantly higher in 75 days cold stored fruit (71.6 N) as compared to 120 days cold stored 'Fuji' fruit (67.9 N) followed by 10 days simulated shelf conditions. Interaction of cold storage periods and fumigation treatments was significant for firmness of 'Fuji' cultivar. After 75 and 120 days CS and 10 days holding at shelf conditions, 1-MCP treated fruit showed significantly highest fruit firmness in 'Fuji' (78.5 and 75.2 N respectively) in comparison to control and all other ethylene antagonists. In 75 days cold stored 'Fuji', the fruit treated with 1-HCP, (*S*)-(-)-limonene and TCA also exhibited significantly firmer fruit (70.1 - 72.9 N) as compared to control (65.6 N). Similarly, after 120 days CS, the fruit firmness in 1-HCP, (*S*)-(-)-limonene and TCA treated 'Fuji' fruit was significantly higher (66.3 - 71.7 N) in comparison to control (56.4 N).

'Cripps Pink' fruit also exhibited significantly highest mean fruit firmness (79.1 N) when treated with 1-MCP as compared to all other fumigation treatments and control (Table 4.5). 1-HCP and TCA treated fruit showed significantly higher mean firmness (57.2 and 55.9 N respectively) as compared to (*S*)-(-)-limonene and control. Storage period means indicated that 120 days cold stored 'Cripps Pink' fruit had significantly higher firmness (59.31 N) than 75 days CS (57.42 N) after 10 days of simulated shelf conditions. Interaction of ethylene antagonist treatments and cold storage periods was found to be significant for fruit firmness in 'Cripps Pink'. 1-MCP treated 'Cripps Pink' fruit showed significantly higher firmness after 75 and 120 days CS (79.5 and 78.8 N) followed by 10 days of simulated shelf conditions in comparison to all other treatments and control (Table 4.5).

Table 4.5. Effect of ethylene antagonists on fruit firmness of apple cv. 'Fuji' and 'Cripps Pink' after 75 and 120 days of cold storage.

Ethylene antagonists	Firmness (N)					
	'Fuji'		Mean (T)	'Cripps Pink'		Mean (T)
	75 d	120 d		75 d	120 d	
Control	65.6±0.09b	56.4±0.07a	60.9a	47.9±0.17b	44.7±0.08a	46.3a
1-MCP	78.5±0.33e	75.2±0.85de	76.9d	79.5±0.58f	78.8±0.15f	79.1d
1-HCP	70.7±0.72c	66.3±0.07b	68.5b	57.4±0.06de	56.9±0.50d	57.2c
(S)-(-)-Lim	70.1±0.39c	69.8±0.34c	69.9bc	49.9±0.14b	56.7±0.23d	53.3b
TCA	72.9±0.58cd	71.7±0.37c	72.3c	52.5±0.08c	59.4±0.39e	55.9c
Mean (SP)	71.6b	67.9a		57.42a	59.31b	
LSD ( $P \leq 0.05$ )	T = 2.4, SP = 1.5, T×SP = 3.4			T = 1.6, SP = 1.1, T×SP = 2.3		

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M), T = treatment, SP = storage period (days), T  $\times$  SP = Interaction of treatments and storage periods.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

**4.3.4. TA, SSC and SSC/TA ratio**

In cultivar 'Fuji', the untreated fruit exhibited significantly lowest mean TA (0.22 %) as compared to all other fumigation treatments ranging from 0.28 to 0.38 %. Mean TA was significantly highest in 1-MCP treated fruit as compared to the control and all other fumigation treatments. The fruit stored for 120 days CS followed by 10 days simulated shelf conditions showed significantly lower mean TA (0.23 %) than 75 days cold stored fruit (0.38 %). The interaction between different treatments and cold storage period for TA was found to be significant. 1-MCP and 1-HCP treated fruit showed significantly higher TA (0.42 and 0.47 % respectively) in 75 days cold stored 'Fuji' fruit as compared to all other treatments and control. Meanwhile, TA was significantly higher in 120 days cold stored fruit treated with 1-MCP and TCA (0.35 and 0.29 % respectively) (Table 4.6).

'Fuji' fruit fumigated with 1-MCP showed significantly highest mean SSC (17.25 %) as compared to all other treatments and control. Mean SSC was significantly higher (16.9 %) in 120 days cold stored fruit than 75 days (16.3 %). The interaction between different ethylene antagonists and cold storage period was also significant. The fruit fumigated with 1-MCP resulted in significantly highest SSC (17.1 and 17.4 %) after 75 and 120 days CS respectively as compared to the control and all other treatments (Table 4.6).

Mean SSC/TA ratio was significantly reduced in cold stored 'Fuji' fruit irrespective of the treatment as compared to the control. 1-HCP fumigated fruit exhibited significantly highest mean SSC/TA ratio (73.75) as compared to all other treatments. Mean SSC/TA ratio was significantly higher in 120 days (82.6) than 75 days CS fruit (44.8). The interaction between treatments and cold storage period was found to be significant for SSC/TA ratio. In 75 days cold stored fruit, SSC/TA was significantly reduced with 1-HCP fumigation (32.7) as compared to control and at par with all other treatments. In 120 days cold stored fruit, SSC/TA ratio was significantly higher in 1-HCP treated fruit (114.7) as compared to all other treatments except control (116.2) (Table 4.6).

In 'Cripps Pink', mean TA was significantly highest in the fruit treated with 1-MCP (0.84 %) followed by TCA (0.72 %) as compared to control and all other treatments (Table 4.7). Mean TA was higher in 75 days cold stored followed by 10 days simulated shelf conditions 'Fuji' fruit (0.68 %) as compared to 120 days stored fruit (0.63 %). Interaction of fumigation treatments and storage periods was significant for TA. Highest TA was noted in 1-MCP treated 'Cripps Pink' fruit after 75 days CS (0.82 %) which was significantly higher than 1-HCP treatment and control but at par with (*S*)-(-)-limonene (0.72 %) and TCA (0.73 %). 1-MCP treated fruit also showed significantly highest TA (0.86 %) after 120 days CS followed by 10 days simulated shelf conditions as compared to the control and all other treatments.

Mean SSC in 'Cripps Pink' fruit was significantly higher in the fruit treated with TCA and (*S*)-(-)-limonene (15.6 and 15.2 %) as compared to 1-MCP and control except 1-HCP (Table 4.7). Among storage periods, SSC was significantly higher in 120 days cold stored fruit followed by 10 days simulated shelf conditions (15.1 %) than 75 days storage (14.2 %). 1-MCP treated 'Cripps Pink' fruit exhibited minimum SSC (9.7 %) after 75 days CS followed by 10 days simulated shelf conditions compared to control and all other ethylene antagonists. SSC was significantly highest (16.0 %) in 1-MCP treatment after 120 days CS followed by 10 days simulated shelf conditions as compared to control and all other treatments except TCA (15.5 %).

Mean SSC/TA ratio was significantly reduced in cold stored 'Cripps Pink' fruit with all the treatments as compared to the control except 1-HCP treated fruit (Table 4.7). Mean SSC/TA was significantly higher in 120 days cold stored fruit (25.1) than 75 days storage followed by 10 days simulated shelf conditions (22.3). There was a significant interaction between treatments and cold storage periods for SSC/TA ratio. All the treatments had significantly reduced SSC/TA ratio (11.9 - 21.9) as compared to the control (34.4) in the 75 days cold stored fruit. In 120 days cold stored fruit followed by 10 days simulated shelf conditions, all the treatments and control have reduced SSC/TA ratio (18.7 - 26.5) except 1-HCP fumigation (33.9).

Table 4.6. Effect of ethylene antagonists on TA, SSC and SSC/TA ratio of apple cv. 'Fuji' after 75 and 120 days of cold storage.

Ethylene antagonists	TA (%)		Mean (T)	SSC (%)		Mean (T)	SSC/TA ratio		Mean (T)
	75 d	120 d		75 d	120 d		75 d	120 d	
Control	0.29±0.01c	0.15±0.01a	0.22a	16.2±0.08b	16.8±0.02d	16.50b	55.1±0.71b	116.2±4.90d	85.63d
1-MCP	0.42±0.01ef	0.35±0.01cd	0.38d	17.1±0.04ef	17.4±0.04f	17.25c	41.3±0.45ab	50.0±0.90ab	45.67a
1-HCP	0.47±0.01f	0.15±0.01a	0.31bc	15.2±0.01a	16.6±0.02cd	15.88a	32.7±1.03a	114.7±4.84d	73.75c
(S)-(-)-Lim	0.34±0.01cd	0.23±0.01b	0.28b	16.4±0.04bc	16.9±0.01de	16.62b	48.9±0.70ab	74.6±1.84c	61.78b
TCA	0.36±0.01de	0.29±0.01c	0.33c	16.5±0.02c	16.9±0.04de	16.68b	46.0±1.28ab	57.4±0.94b	51.7ab
Mean (SP)	0.38b	0.23a		16.3a	16.9b		44.8a	82.6b	
LSD ( $P \leq 0.05$ )	T = 0.04, SP = 0.02, T × SP = 0.06			T = 0.19, SP = 0.12, T × SP = 0.27			T = 11.8, SP = 7.5, T × SP = 16.7		

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M), T = treatment, SP = storage period (days), T × SP = Interaction of treatments and storage periods.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 4.7. Effect of ethylene antagonists on TA, SSC and SSC/TA ratio of apple cv. 'Cripps Pink' after 75 and 120 days of cold storage.

Ethylene antagonists	TA (%)		Mean (T)	SSC (%)		Mean (T)	SSC/TA ratio		Mean (T)
	75 d	120 d		75 d	120 d		75 d	120 d	
Control	0.44±0.01a	0.58±0.01b	0.51a	15.1±0.04c	14.4±0.14b	14.9b	34.4±0.70e	25.1±0.04cd	29.7c
1-MCP	0.82±0.01df	0.86±0.01f	0.84d	9.7±0.02a	16.0±0.15d	12.9a	11.9±0.10a	18.7±0.25b	15.3a
1-HCP	0.69±0.01c	0.43±0.01a	0.56a	15.2±0.04c	14.5±0.07b	14.8bc	21.9±0.32bc	33.9±0.47e	27.9c
(S)-(-)-Lim	0.72±0.02cd	0.58±0.01b	0.65b	15.3±0.04c	15.1±0.12c	15.2cd	21.3±0.47bc	26.5±0.83d	23.9b
TCA	0.73±0.02cd	0.74±0.01cde	0.72c	15.6±0.01cd	15.5±0.07cd	15.6d	21.8±0.56bc	21.5±0.18bc	21.7b
Mean (SP)	0.68b	0.63a		14.2a	15.1b		22.3a	25.1b	
LSD ( $P \leq 0.05$ )	T = 0.06, SP = 0.04, T×SP = 0.09			T = 0.4, SP = 0.3, T×SP = 0.6			T = 2.5, SP = 1.5, T×SP = 3.6		

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M), T = treatment, SP = storage period (days), T × SP = Interaction of treatments and storage periods.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

#### **4.3.5. Individual and Total Sugars**

Three individual sugars *viz.*, sucrose, glucose, fructose and total sugars were quantified in both 'Fuji' (Table 4.8) and 'Cripps Pink' (Table 4.9) apple fruit following 75 and 120 days cold storage with subsequent 10 days holding on simulated shelf conditions.

Mean sucrose concentration was significantly higher in 1-MCP, 1-HCP and TCA treated 'Fuji' fruit (35.6, 36.9 and 36.47 g Kg<sup>-1</sup> respectively) as compared to the control and those treated with (S)-(-)-limonene (Table 4.8). Mean sucrose level was significantly higher in 120 days cold stored 'Fuji' than 75 days CS with subsequent 10 days shelf conditions. The interaction between cold storage periods and fumigation treatments was found to be significant for sucrose levels in 'Fuji'. In 75 days cold stored fruit, significantly highest concentration of sucrose was recorded with 1-HCP (29.9 g Kg<sup>-1</sup>) and TCA (27.3 g Kg<sup>-1</sup>) treatments; while after 120 days CS, sucrose concentration was significantly highest with all the fumigation treatments (43.5 - 46.2 g Kg<sup>-1</sup>) other than control 'Fuji' fruit.

Mean concentrations of glucose, fructose and total sugars were significantly highest with TCA treatment (33.7, 90.8 and 160.9 g Kg<sup>-1</sup>) in 'Fuji' fruit compared to all other treatments and control (Table 4.8). Effect of cold storage periods on the levels of glucose was non-significant in 'Fuji' fruit, however, the mean concentration of fructose was significantly higher in 75 days cold stored fruit (83.9 g Kg<sup>-1</sup>) as compared to 120 days cold stored fruit followed by subsequent 10 days shelf storage. However, significantly higher mean total sugars were noted in 120 days cold stored 'Fuji' fruit (142.7 g Kg<sup>-1</sup>) than 75 days CS. The interaction between treatments and cold storage periods was found to be significant for fructose, glucose and total sugars. When sugars were analysed after 75 days CS of 'Fuji' with 10 days simulated shelf storage, significantly higher concentrations of glucose (38.57 g Kg<sup>-1</sup>), fructose (107.47 g Kg<sup>-1</sup>) and total sugars (173.27 g Kg<sup>-1</sup>) were recorded in TCA treated fruit as compared to all other treatments and control. After 120 days CS, fructose level was significantly higher in control (74.92 g Kg<sup>-1</sup>), (S)-(-)-limonene (75.27 g Kg<sup>-1</sup>) and TCA (74.15 g Kg<sup>-1</sup>) treatments as compared to 1-MCP and 1-HCP. However, total sugars were significantly highest in TCA treated 'Fuji' fruit (148.6 g Kg<sup>-1</sup>) after 120 days CS with 10 days

simulated shelf storage. In general, the concentration of fructose was higher than glucose and sucrose in 'Fuji' irrespective of CS period (Table 4.8).

In 'Cripps Pink', mean concentration of sucrose was significantly highest in 1-HCP and TCA treated fruit (42.9 and 39.7 g Kg<sup>-1</sup>) as compared to the control and all other treatments (Table 4.9). Mean concentration of sucrose was significantly higher in 75 days cold stored fruit (41.5 g Kg<sup>-1</sup>) as compared to 120 days CS (27.5 g Kg<sup>-1</sup>). There was a significant interaction between fumigation treatments and cold storage periods for sucrose level in 'Cripps Pink'. In 75 days cold stored 'Cripps Pink' followed by 10 days simulated shelf conditions, significantly higher concentration of sucrose was noted in the fruit treated with 1-HCP (54.2 g Kg<sup>-1</sup>) and TCA (49.8 g Kg<sup>-1</sup>), whilst in 120 days cold stored fruit which were treated with (*S*)-(-)-limonene, significantly lowest level of sucrose (21.5 g Kg<sup>-1</sup>) was found as compared to all other treatments and control.

Mean glucose concentration of 'Cripps Pink' fruit was significantly higher in the fruit treated with 1-HCP, (*S*)-(-)-limonene and TCA treatments (7.6, 7.1 and 6.8 g Kg<sup>-1</sup> respectively) as compared to the 1-MCP treatment and control (Table 4.9). Mean glucose levels were significantly higher in 75 days cold stored 'Cripps Pink' (11.7 g Kg<sup>-1</sup>) than 120 days cold stored fruit (1.8 g Kg<sup>-1</sup>) after 10 days of simulated shelf storage. The interaction between treatments and cold storage periods was found to be significant for glucose. 1-HCP treated fruit showed significantly highest level of glucose (13.8 g Kg<sup>-1</sup>) after 75 days CS in comparison to all other treatments and control. However, after 120 days CS, none of the treatments significantly influenced the levels of glucose in 'Cripps Pink'.

Mean fructose and total sugars were significantly highest in 1-MCP (105.7 and 148.1 g Kg<sup>-1</sup> respectively) and TCA (98.9 and 145.4 g Kg<sup>-1</sup>) treated fruit as compared to various other fumigation treatments and control (Table 4.9). Mean levels of sucrose and total sugars were significantly higher (132.3 and 161.5 g Kg<sup>-1</sup> respectively) after 120 days CS plus 10 days simulated shelf storage as compared to 75 days cold stored fruit. There was significant interaction between fumigation treatments and cold storage periods for fructose and total sugars in 'Cripps Pink'. 1-MCP treated fruit resulted in lowest concentration of fructose after 75 days CS; whilst after 120 days CS followed by 10 days simulated shelf conditions, 1-MCP fumigation resulted in significantly highest concentration of fructose as compared to all other treatments and control. On the other

hand, significant increase in total sugars was observed in the fruit treated with 1-HCP (121.9 g Kg<sup>-1</sup>) and TCA (112.2 g Kg<sup>-1</sup>) after 75 days CS, while 120 days cold stored 'Cripps Pink' showed significantly highest total sugars when treated with 1-MCP (206.8 g Kg<sup>-1</sup>).

Table 4.8. Effect of ethylene antagonists on individual and total sugars of apple cv. 'Fuji' after 75 and 120 days of cold storage.

Ethylene antagonists	Sugars (g kg <sup>-1</sup> )					
	Sucrose		Mean (T)	Glucose		Mean (T)
	75 d	120 d		75 d	120 d	
Control	21.3±0.1a	40.0±0.4e	30.7a	26.2±0.0cd	28.2±0.0d	27.2c
1-MCP	25.1±0.0bc	46.2±0.2f	35.6b	26.9±0.1cd	28.5±0.7d	27.7c
1-HCP	29.9±0.8d	44.0±0.2f	36.9b	22.6±0.1b	26.0±0.0cd	24.3b
(S)-(-)-Lim	22.1±0.3ab	43.5±0.8f	32.8a	17.1±0.3a	24.6±0.8bc	20.9a
TCA	27.3±0.1cd	45.5±0.2f	36.4b	38.5±0.1e	28.9±0.1d	33.7d
Mean (SP)	25.1a	43.9b		26.3	27.3	
	Fructose		Mean (T)	Total Sugars		Mean (T)
	75 d	120 d		75 d	120 d	
	Control	79.3±0.1c	74.9±0.2b	77.1c	126.8±0.0b	143.2±0.1f
1-MCP	81.3±0.4c	66.6± 0.1a	73.9b	133.2± 0.5c	141.3±0.6e	137.2bc
1-HCP	86.4±0.3d	67.1± 0.1a	76.7c	138.9±1.0de	137.2±0.3d	138.0c
(S)-(-)-Lim	65.6±0.7a	75.3±0.6b	70.5a	104.8± 0.7a	143.5±0.6f	124.1a
TCA	107.4±0.1e	74.2± 0.0b	90.8d	173.2± 0.3h	148.6±0.1g	160.9d
Mean (SP)	83.9b	71.6a		135.4a	142.7b	

LSD at  $P \leq 0.05$  for sucrose; T = 2.2, SP = 1.4, T × SP = 3.1, for glucose; T = 1.9, SP = NS, T × SP = 2.8, for fructose; T = 1.8, SP = 1.1, T × SP = 2.6 and for total Sugars; T = 2.6, SP = 1.7, T × SP = 3.7.

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 μM), (S)-(-)-Lim = (S)-(-)-limonene (1 μM), TCA = *trans*-cinnamaldehyde (1 μM), T = treatment, SP = storage period (days), T × SP = Interaction of treatments and storage periods, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 4.9. Effect of ethylene antagonists on individual and total sugars of apple cv. 'Cripps Pink' after 75 and 120 days of cold storage.

Ethylene antagonists	Sugars (g kg <sup>-1</sup> )					
	Sucrose		Mean (T)	Glucose		Mean (T)
	75 d	120 d		75 d	120 d	
Control	24.8a±0.8b	24.5ab	24.7a	9.8± 0.1b	1.8±0.0a	5.8a
1-MCP	41.9±1.4d	30.0b	35.9c	10.5±0.4b	2.4±0.0a	6.5ab
1-HCP	54.2±1.5e	31.5bc	42.9d	13.8±0.0d	1.3±0.0a	7.6c
(S)-(-)-Lim	36.9±0.6cd	21.5a	29.2b	12.3±0.1c	1.9±0.0a	7.1bc
TCA	49.8±0.7e	29.7b	39.7cd	12.2±0.3c	1.4±0.0a	6.8bc
Mean (SP)	41.5b	27.5a		11.7b	1.8a	
	Fructose		Mean (T)	Total Sugars		Mean (T)
	75 d	120 d		75 d	120 d	
	Control	44.1±0.2ab	117.8±0.3	80.9a	78.7±1.1a	144.1±1.2e
1-MCP	36.9±0.4a	174.4±2.3	105.7b	89.4±0.8ab	206.8±2.2 g	148.1c
1-HCP	53.9±2.8b	112.0±1.0	82.9a	121.9±4.1c	144.8±1.1e	133.4b
(S)-(-)-Lim	45.6±0.1ab	109.6±0.7	77.6a	94.9± 0.4b	132.9±0.4d	113.9a
TCA	50.2±1.0b	147.6±2.9	98.9b	112.2± 0.8c	178.7±2.8f	145.4c
Mean (SP)	46.1a	132.3b		99.4a	161.5b	

LSD at  $P \leq 0.05$  for sucrose; T = 4.6, SP = 2.9, T × SP = 6.5, for glucose; T = 0.8, SP = 0.5, T × SP = 1.2, for fructose; T = 7.5, SP = 4.7, T × SP = 10.6 and for total Sugars; T = 9.4, SP = 5.9, T × SP = 13.3.

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 μM), (S)-(-)-Lim = (S)-(-)-limonene (1 μM), TCA = *trans*-cinnamaldehyde (1 μM), T = treatment, SP = storage period (days), T × SP = Interaction of treatments and storage periods, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

### **4.3.6. Individual and Total Organic Acids**

Three different individual organic acids *viz.*, malic, succinic and fumaric acid were quantified in 'Fuji' apple (Table 4.10) while in 'Cripps Pink' fruit, malic, succinic and citric acid were identified (Table 4.11). Total organic acids were calculated in both cultivars by totalling concentrations of all individual organic acids.

Mean concentration of malic acid was significantly highest in 1-MCP treated 'Fuji' fruit (2.8 g Kg<sup>-1</sup>) as compared to all other treatments and control (Table 4.10). A significant interaction between fumigation treatments and storage periods was observed for malic acid. After 75 days CS and 10 days simulated shelf conditions, 'Fuji' fruit treated with 1-MCP and 1-HCP resulted in significantly highest concentration of malic acid (3.23 and 2.56 g Kg<sup>-1</sup> respectively) as compared to control and all other treatments. After 120 days CS, TCA treated fruit showed significantly lowest levels of malic acid as compared to control and all other treatments.

All the treatments did not significantly affect the concentration of fumaric acid in 'Fuji' fruit stored either for 75 or 120 days (Table 4.10). The interaction between treatments and cold storage period was also found to be non-significant for level of fumaric acid. Mean level of fumaric acid was significantly higher (0.30 g Kg<sup>-1</sup>) in 120 days cold stored 'Fuji' followed by 10 days simulated shelf storage than 75 days cold stored fruit (0.26 g Kg<sup>-1</sup>).

Mean concentration of succinic acid in 'Fuji' fruit was significantly reduced by all the treatments (0.14-0.49 g Kg<sup>-1</sup>) in comparison to control (0.78 g Kg<sup>-1</sup>) (Table 4.10). Mean total organic acids of 'Fuji' cultivar was significantly highest in control (3.05 g Kg<sup>-1</sup>) and 1-MCP (3.55 g Kg<sup>-1</sup> respectively) treated fruit as compared to all other treatments. Effect of storage periods was non-significant for succinic acid in 'Fuji', nonetheless; mean concentration of total organic acids was significantly higher after 75 days CS (2.85 g Kg<sup>-1</sup>) than 120 days CS (2.30 g Kg<sup>-1</sup>). Interaction of fumigation treatments and storage periods was significant for both succinic acid and total organic acids. All the fumigation treatments have significantly reduced the level of succinic acid in 'Fuji' after 75 and 120 days CS with subsequent 10 days simulated shelf storage in comparison to control. Fumigation with TCA significantly reduced the concentration of total organic acids in 'Fuji' fruit in comparison to all other treatments

and control, when cold stored for 75 and 120 days followed by 10 days simulated shelf conditions.

In 'Cripps Pink', mean malic acid concentration was significantly reduced by all the fumigation treatments in comparison to control (7.51 g Kg<sup>-1</sup>) and TCA (6.91 g Kg<sup>-1</sup>) treated fruit (Table 4.11). Mean malic acid level was significantly higher in 75 days cold stored 'Cripps Pink' (7.27 g Kg<sup>-1</sup>) than 120 days CS (5.43 g Kg<sup>-1</sup>). Interaction of treatments and cold storage periods was found to be significant for malic acid in 'Cripps Pink'. In 75 days cold stored fruit, fumigation treatments significantly reduced the malic acid concentration (5.92-7.11 g Kg<sup>-1</sup>) than control (9.65 g Kg<sup>-1</sup>). However, after 120 days CS, malic acid was significantly higher in TCA and 1-MCP treated fruit (7.06 and 5.63 g Kg<sup>-1</sup> respectively) (Table 4.11).

Mean succinic acid concentration was significantly reduced with all fumigation treatments as compared to 1-MCP treatment and control (0.46 and 0.38 g Kg<sup>-1</sup>). Among cold storage periods, mean succinic acid was significantly higher in 120 days cold stored fruit (0.39 g Kg<sup>-1</sup>) than 75 days CS (0.17 g Kg<sup>-1</sup>). The levels of citric acid and total organic acids were not significantly affected by any of the treatments (Table 4.11).

Table 4.10. Effect of ethylene antagonists on individual and total organic acids of apple cv. 'Fuji' after 75 and 120 days of cold storage.

Ethylene antagonists	Organic acids (g kg <sup>-1</sup> )					
	Malic acid		Mean (T)	Succinic acid		Mean (T)
	75 d	120 d		75 d	120 d	
Control	1.99±0.08bc	1.96±0.07bc	1.97b	0.79±0.0e	0.77±0.0e	0.78d
1-MCP	3.23±0.12d	2.35±0.29bc	2.80c	0.57±0.1d	0.42±0.0cd	0.49c
1-HCP	2.56±0.18cd	1.26±0.16ab	1.90b	0.25±0.0bc	0.05±0.0a	0.14a
(S)-(-)-Lim	1.92±0.05bc	2.01±0.16bc	1.96b	0.30±0.0bc	0.20±0.0ab	0.25ab
TCA	1.22±0.03ab	0.55±0.01a	0.89a	0.15±0.0ab	0.41±0.0cd	0.28b
Mean (SP)	2.18b	1.63a		0.41	0.37	
	Fumaric acid		Mean (T)	Total organic acids		Mean (T)
	75 d	120 d		75 d	120 d	
	Control	0.256±0.0	0.329±0.0	0.29	3.0±0.0cd	3.1±0.1cd
1-MCP	0.259±0.0	0.277±0.0	0.27	4.1±0.2d	3.0±0.3 cd	3.55c
1-HCP	0.257±0.0	0.323±0.0	0.29	3.1±0.2cd	1.6±0.2ab	2.35b
(S)-(-)-Lim	0.261±0.0	0.267±0.0	0.26	2.5±0.1abc	2.5±0.2bc	2.48b
TCA	0.259±0.0	0.303±0.0	0.28	1.63±0.0ab	1.3±0.0a	1.45a
Mean (SP)	0.26a	0.30b		2.85b	2.30a	

LSD at  $P \leq 0.05$  for malic acid;  $T = 0.7$ ,  $SP = 0.5$ ,  $T \times SP = 1.0$ , for succinic acid;  $T = 0.12$ ,  $SP = NS$ ,  $T \times SP = 0.17$ , for fumaric acid;  $T = NS$ ,  $SP = 0.03$ ,  $T \times SP = NS$  and for total organic acids;  $T = 0.7$ ,  $SP = 0.5$ ,  $T \times SP = 1.2$ .

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1µM), (S)-(-)-Lim = (S)-(-)-limonene (1µM), TCA = *trans*-cinnamaldehyde (1µM), T = treatment, SP = storage period (days),  $T \times SP$  = Interaction of treatments and storage periods, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 4.11. Effect of ethylene antagonists on individual and total organic acids of apple cv. 'Cripps Pink' after 75 and 120 days of cold storage.

Ethylene antagonists	Organic acids (g kg <sup>-1</sup> )					
	Malic acid		Mean (T)	Succinic acid		Mean (T)
	75 d	120 d		75 d	120 d	
	Citric acid		Mean (T)	Total organic acids		Mean (T)
75 d	120 d	75 d		120 d		
Control	9.65±0.5d	5.37±0.2ab	7.51d	0.26±0.1	0.51±0.0	0.38bc
1-MCP	7.11±0.3c	5.63±0.3abc	6.37bc	0.25±0.0	0.68±0.1	0.46c
1-HCP	6.87±0.0c	4.58±0.1a	5.72ab	0.07±0.0	0.16±0.0	0.11a
(S)-(-)-Lim	5.92±0.3abc	4.51±0.2a	5.22a	0.19±0.0	0.27±0.0	0.23ab
TCA	6.78±0.2bc	7.06±0.1c	6.91cd	0.09±0.0	0.35±0.1	0.22ab
Mean (SP)	7.27b	5.43a		0.17a	0.39b	
Control	5.69±1.2	12.4±0.5	9.06	15.85±1.1	18.57±0.3	17.21
1-MCP	11.06±0.8	9.60±0.9	10.32	18.68±0.3	16.16±0.5	17.42
1-HCP	13.58±0.3	11.79±1.1	12.68	20.77±0.4	16.78±1.2	18.77
(S)-(-)-Lim	8.38±0.3	11.59±0.7	9.98	14.75±1.0	16.62±0.6	15.69
TCA	4.50±0.7	9.24±0.7	6.87	11.63±0.7	16.90±0.8	14.27
Mean (SP)	8.64	10.9		16.34	17.01	

LSD at  $P \leq 0.05$  for malic acid; T = 0.9, SP = 0.6, T × SP = 1.3, for succinic acid; T = 0.2, SP = 0.1, T × SP = NS, for citric acid; T = NS, SP = NS, T × SP = NS and for total organic acids; T = NS, SP = NS, T × SP = NS.

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 μM), (S)-(-)-Lim = (S)-(-)-limonene (1 μM), TCA = *trans*-cinnamaldehyde (1 μM), T = treatment, SP = storage period (days), T × SP = Interaction of treatments and storage periods, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

**4.3.7. Ascorbic Acid**

Fumigation treatments of 1-MCP and (*S*)-(-)-limonene resulted in significantly higher mean concentration of ascorbic acid in 'Fuji' fruit (365.0 and 342.9 mg Kg<sup>-1</sup> respectively) as compared to all other fumigation treatments and control (Table 4.12). Comparison of cold storage means exhibited a significantly higher level of ascorbic acid in 75 days cold stored 'Fuji' (330.5 mg Kg<sup>-1</sup>) as compared to 120 days cold stored fruit (301.8 mg Kg<sup>-1</sup>) following 10 days holding at simulated shelf conditions. Fumigation treatments and cold storage periods exhibited a significant interaction for ascorbic acid in 'Fuji' fruit. 1-MCP and (*S*)-(-)-limonene treated fruit showed significantly highest concentration of ascorbic acid (373.4 and 354.2 mg Kg<sup>-1</sup> respectively) following 75 days CS in comparison to all other treatments and untreated fruit. Likewise, in 120 days cold stored 'Fuji' fruit, 1-MCP fumigation resulted in significantly highest ascorbic acid concentration (356.6 mg Kg<sup>-1</sup>) as compared to all other fumigation treatments and control.

In 'Cripps Pink' fruit, mean concentration of ascorbic acid was significantly highest in 1-MCP treated fruit (323.6 mg Kg<sup>-1</sup>) as compared to other ethylene antagonists and control (Table 4.13). 1-HCP, (*S*)-(-)-limonene and TCA treated fruit showed significantly higher levels of ascorbic acid as compared to control. Mean ascorbic acid concentration was significantly higher in 75 days cold stored 'Cripps Pink' fruit (297.5 mg Kg<sup>-1</sup>) than 120 days CS (256.8 mg Kg<sup>-1</sup>) following 10 days holding at simulated shelf conditions. There was a significant interaction between ethylene antagonist treatments and cold storage intervals for ascorbic acid in 'Cripps Pink'. 1-MCP and TCA treated 'Cripps Pink' fruit exhibited significantly highest ascorbic acid concentration (342.0 and 311.5 mg Kg<sup>-1</sup> respectively) as compared to control and all other fumigation treatments after 75 days CS following 10 days holding at ambient conditions. 1-MCP treatment resulted in significantly highest level of ascorbic acid (305.1 mg Kg<sup>-1</sup>) in 120 days cold stored fruit in comparison to all other treatments and untreated fruit (Table 4.13).

**4.3.8. Total Phenolics**

Concentration of total phenolics in 'Fuji' cultivar was not significantly affected by the treatments, cold storage periods and by the interaction of treatments and cold storage periods (Table 4.12).

Mean total phenolic concentration was significantly highest in 1-MCP treated 'Cripps Pink' fruit (5133 mg Kg<sup>-1</sup> GAE) as compared to all other treatments and control fruit (Table 4.13). The levels of mean total phenolics were significantly higher in 75 days cold stored fruit (4667 mg Kg<sup>-1</sup> GAE) than 120 days CS (4280 mg Kg<sup>-1</sup> GAE) after 10 days of simulated shelf conditions. Interaction of treatments and cold storage periods was found to be significant for total phenolics in 'Cripps Pink'. In 75 days cold stored 'Cripps Pink' fruit, 1-MCP, 1-HCP and (S)-(-)-limonene treatments resulted in significantly higher concentration of total phenolics (4967, 4767 and 4933 mg Kg<sup>-1</sup> GAE respectively) as compared to TCA fumigation treatment and control. 1-MCP treatment resulted in significantly increased concentration of total phenolics (5300 mg Kg<sup>-1</sup> GAE) in 120 days cold stored fruit after 10 days of simulated shelf conditions in comparison to all other treatments and control. 1-HCP, (S)-(-)-limonene and TCA treatments also produced significantly higher total phenolics (3933-4333 mg Kg<sup>-1</sup> GAE) compared to control after 120 days CS and were parallel to each other (Table 4.13).

#### **4.3.9. Total Antioxidants**

Mean total antioxidants in 'Fuji' fruit were significantly higher in the cold stored fruit which were treated with TCA (167.2 mM Trolox kg<sup>-1</sup>) and 1-MCP (159.1 mM Trolox kg<sup>-1</sup>) as compared to all other treatments and control (Table 4.12). Mean total antioxidants were significantly higher after 75 d CS (164.28 mM Trolox kg<sup>-1</sup>) than 120 days cold stored (110.76 mM Trolox kg<sup>-1</sup>) 'Fuji' fruit after 10 days of simulated shelf storage. The interaction between treatments and storage period was found to be significant for total antioxidants. After 75 days CS, significantly highest total antioxidant concentration was recorded in TCA treated 'Fuji' fruit (211.3 mM Trolox kg<sup>-1</sup>), while after 120 days CS, 1-MCP treated fruit showed significantly higher total antioxidants (135.6) as compared to all other treatments and control.

In 'Cripps Pink', significantly increased mean total antioxidants were exhibited by TCA fumigated fruit (175.4 mM Trolox kg<sup>-1</sup>) in comparison to all other treatments and control (Table 4.13). Significantly higher concentration of mean total antioxidants was noted in 75 days cold stored fruit (147.4 mM Trolox kg<sup>-1</sup>) than 120 days cold stored fruit (130.8 mM Trolox kg<sup>-1</sup>). There was a significant interaction between different fumigation treatments and cold storage periods for antioxidant capacity in 'Cripps Pink'. After 75 days CS and subsequent 10 days simulated shelf storage, TCA treated fruit resulted in

significantly highest antioxidant capacity (191.5 mM Trolox kg<sup>-1</sup>) in 'Cripps Pink' when compared with other fumigation treatments and control. 1-MCP and (*S*)-(-)-limonene treated fruit resulted in significantly higher levels of antioxidants (144.3 and 140.7 mM Trolox kg<sup>-1</sup> respectively) in comparison to those treated with 1-HCP and control. In 120 days cold stored 'Cripps Pink', 1-MCP and TCA fumigation treatments resulted in significantly higher levels of antioxidants (168.5 and 159.3 mM Trolox kg<sup>-1</sup> respectively) compared to all other fumigation treatments and control (Table 4.13).

Table 4.12. Effect of ethylene antagonists on ascorbic acid, total phenolics and total antioxidants of apple cv. 'Fuji' after 75 and 120 days of cold storage.

Ethylene antagonists	Ascorbic acid (mg Kg <sup>-1</sup> )		Mean (T)	Total phenolics (GAE mg kg <sup>-1</sup> )		Mean (T)	Total antioxidants (mM Trolox kg <sup>-1</sup> )		Mean (T)
	75 d	120 d		75 d	120 d		75 d	120 d	
Control	280.3±1.41bc	244.0±0.39a	262.1a	3767±370.4	3867±36.0	3817	107.5±0.0b	92.9±2.8a	100.2a
1-MCP	373.4±1.85g	356.6±4.71fg	365.0c	4800±227.6	4200±23.6	4500	182.7±0.2g	135.6±0.4d	159.1d
1-HCP	317.8±2.73de	269.9±9.25ab	293.8b	4300±232.4	4200±40.9	4250	153.7±0.4e	85.8±0.1a	119.7b
(S)-(-)-Lim	354.2±1.49efg	331.6±2.06def	342.9c	4300±165.2	4500±85.1	4400	166.1±0.1f	116.5±0.0bc	141.3c
TCA	326.6±2.71def	306.8±9.47cd	316.7b	4833±89.3	4033±13.6	4433	211.3±3.9h	122.9±1.3c	167.2d
Mean (SP)	330.5b	301.8a		4400	4160		164.28b	110.76a	
LSD ( $P \leq 0.05$ )	T = 24, SP = 15.2, T × SP = 33.9			T = NS, SP = NS, T × SP = NS			T = 8.2, SP = 5.2, T × SP = 11.6		

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = treatment, SP = storage period (days), T × SP = Interaction of treatments and storage periods. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 4.13. Effect of ethylene antagonists on ascorbic acid, total phenolics and total antioxidants of apple cv. 'Cripps Pink' after 75 and 120 days of cold storage.

Ethylene antagonists	Ascorbic Acid (mg Kg <sup>-1</sup> )		Mean (T)	Total Phenolics (GAE mg kg <sup>-1</sup> )		Mean (T)	Total Antioxidants (mM Trolox kg <sup>-1</sup> )		Mean (T)
	75 d	120 d		75 d	120 d		75 d	120 d	
Control	236.0±3.0b	192.2±1.7a	214.1a	4300±40.9bc	3500±0.0a	3900a	125.1±0.26c	101.8±2.64b	113.4a
1-MCP	342.0±9.6e	305.1±1.8d	323.6c	4967±13.6cd	5300±178.2d	5133c	144.3±0.17d	168.5±0.70e	156.4c
1-HCP	291.1±7.1cd	255.0±1.3b	273.0b	4767±27.2cd	3933±151.7ab	4350b	135.5±0.32cd	81.1±0.39a	108.3a
(S)-(-)-Lim	306.8±2.6d	264.6±1.7bc	285.7b	4933±13.6cd	4333±49.1bc	4633b	140.7±0.28d	143.3±0.52d	142.0b
TCA	311.5±3.9de	266.8±2.5bc	289.1b	4367±106.4bc	4333±36.0bc	4350b	191.5±1.26f	159.3±3.88e	175.4d
Mean (SP)	297.5b	256.8a		4667b	4280a		147.4b	130.8a	
LSD ( $P \leq 0.05$ )	T = 22.2, SP = 14.0, T × SP = 31.5			T = 436.8, SP = 276.2, T × SP = 617.7			T = 8.3, SP = 5.2, T × SP = 11.8		

1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1µM), (S)-(-)-Lim = (S)-(-)-limonene (1µM), TCA = *trans*-cinnamaldehyde (1µM), T = treatment, SP = storage period (days), T × SP = Interaction of treatments and storage periods. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

**4.4. Discussion**

1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA fumigated 'Fuji' and 'Cripps Pink' fruit following cold storage showed significantly suppressed and delayed climacteric ethylene peaks as compared to the control. Amongst all ethylene antagonists tested, 1-MCP was more effective as compared to 1-HCP, (*S*)-(-)-limonene and TCA in delaying and suppressing climacteric ethylene peaks in cold stored fruit depending upon storage periods and cultivar.

Reduced ethylene production in 1-MCP treated apples has also been previously reported (Watkins, 2008; Lee *et al.*, 2012; Lu *et al.*, 2013a) describing that 1-MCP application has suppressed ethylene production and action in climacteric fruits. 1-MCP has also been reported to down-regulate the activity of ACS (1-aminocyclopropane-1-carboxylate synthase) and ACO (1-aminocyclopropane-1-carboxylate oxidase), which are the key enzymes involved in ethylene biosynthesis (Bulens *et al.*, 2011). In addition, the accumulation of transcripts for these enzymes in plant tissues was also decreased in 1-MCP treated apple fruit of Cortland, Law Rome, and Idared cultivars (Tsantili *et al.*, 2007). Possibly, both factors may have resulted in highly suppressed and delayed climacteric ethylene peaks in cold stored apple fruit fumigated with 1-MCP. Delayed and suppressed climacteric ethylene peaks with fumigation of 1-HCP, (*S*)-(-)-limonene and TCA may be ascribed to the blockage of ethylene receptor sites irreversibly consequently inhibiting ethylene action. Reduction in the transcripts of genes which are responsible for the signal transduction of ethylene i.e., ETR1 and ERS1 could also be a factor (Dal Cin *et al.*, 2006; Munoz-Robredo *et al.*, 2012). Period of cold storage has also greatly affected the suppression of climacteric ethylene peaks and their delay in both cultivars. In general, the concentration of ethylene in both cultivars increased considerably with extension of cold storage periods; while the number of days to approach climacteric peak were reduced.

All fumigation treatments were effective in suppressing climacteric respiration peaks in cold stored 'Fuji' and 'Cripps Pink' apple fruit as compared to the control. 1-MCP fumigation was most effective followed by 1-HCP, (*S*)-(-)-limonene and TCA fumigation in cold stored fruit in suppressing respiratory peaks as compared to the control. However, 1-MCP, (*S*)-(-)-limonene and TCA fumigation were equally effective in delaying respiratory climacteric as compared to 1-HCP and control in both cultivars.

Respiratory climacteric in both apple cultivars was also remarkably influenced by storage intervals.

Reduced rate of respiration in both cultivars and delayed respiratory climacteric in 'Cripps Pink' primarily by 1-MCP and to some extent by TCA and (*S*)-(-)-limonene can probably be attributed to the low levels of ethylene produced by these treatments as ethylene is closely associated with rate of respiration (Ullah *et al.*, 2016). This can also be explained contrariwise that low rate of respiration caused by these ethylene antagonists is responsible for reducing climacteric ethylene as suppressed respiration leads to a decline in ATP production, which is required for the conversion of ACC to ethylene (de Wild *et al.*, 1999).

It has also been reported earlier by Duque and Arrabaca (1999) that the climacteric burst in respiration is reduced by cold storage below 5 degrees Celsius in apples which supports the respiratory behaviour of cold stored 'Fuji' and 'Cripps Pink' in this study. Holding fruit at ambient conditions after cold storage increased the respiration rate in control fruit of both cultivars as with every 10 °C rise, metabolic processes increase 2-3 times (Saltveit, 2016).

Apple firmness is a significant attribute of fruit quality determining consumer acceptance and loss of fruit firmness has been associated with modifications in the cell wall during the ripening process (Costa *et al.*, 2012). Fumigation of all ethylene antagonists was effective in maintaining fruit firmness following cold storage as compared to the control in both cultivars. 1-MCP fumigation was most effective followed by the fumigation of TCA, (*S*)-(-)-limonene and 1-HCP in maintaining fruit firmness in both cultivars following cold storage. The reduction in loss of fruit firmness may be ascribed to the delayed and reduced climacteric ethylene production with fumigation of these ethylene antagonists. This hypothesis is supported in an earlier study by Watkins *et al.* (2000) who reported that fruit softening in apple is induced by climacteric ethylene which must be present continuously at required levels to initiate fruit softening.

Positive effect of 1-HCP, (*S*)-(-)-limonene and TCA to reduce the loss of firmness after cold storage in comparison to control can be referred to their varying levels of ethylene inhibitory action causing reduction in the activity of pectin degrading enzymes (PME) Lohani *et al.* (2004). 1-HCP has also been reported to be a valuable ethylene antagonist to maintain fruit quality in avocado and tomato by Apelbaum *et al.* (2008) and

Khan *et al.* (2016). Carvalho *et al.* (2016) validates by histological assay that TCA fortified fruit coating in melon reduced the respiration rate and consequent loss of turgor and cell wall loosening leading to structural integrity. Contrarily, considerable loss of firmness in control fruit after prolonged storage is due to the higher activity of cell wall hydrolases by climacteric ethylene during ripening in apple which results in pectin solubilisation and ultimate fruit softening (Lohani *et al.*, 2004). Retention of apple fruit firmness by ethylene antagonists has been reported earlier in several cultivars (Saftner *et al.*, 2003; Oraguzie *et al.*, 2007).

Firmness declined in fruit fumigated with ethylene antagonists following 120 days cold storage possibly due to the fact that new ethylene receptors continue to be synthesized during storage nullifying the effect of 1-MCP gradually (Liu *et al.*, 2005). Comparatively, higher firmness of 'Fuji' fruit than 'Cripps Pink' receiving the same fumigation treatments and storage conditions suggest that firmness is mainly a genetic potential varying greatly with strains and cultivars (DeEll *et al.*, 2001).

Soluble solids and fruit acidity (TA) play a key role in the taste of apple fruit and maintenance of these parameters is a great challenge (Fellman *et al.*, 2003). Both 'Fuji' and 'Cripps Pink' had significantly higher acidity levels in the fruit treated with 1-MCP followed by TCA and (*S*)-(-)-limonene treatments. Lurie and Klein (1990) previously claimed that there is probably a connection between declined titratable acidity and increased respiration rate in fruits as organic acids which are responsible for acidity are utilized as substrate for respiration, ultimately resulting in decreased acidity. Therefore, relatively higher acidity levels in 1-MCP, TCA and (*S*)-(-)-limonene treated fruit can possibly be ascribed to reduced respiration rate by these ethylene antagonists and vice versa. SSC percentage of 'Fuji' was significantly higher in 1-MCP treated fruit while in 'Cripps Pink', significantly higher SSC was recorded in TCA and (*S*)-(-)-limonene treatments. Level of SSC in control 'Fuji' fruit was parallel to TCA and (*S*)-(-)-limonene treated fruit whereas, SSC percentage of 1-MCP treated 'Cripps Pink' fruit was at par with control fruit. These observations suggest that inhibition of ethylene action is not essentially related with accumulation of soluble solids as both ethylene-dependent and independent ripening processes exist in climacteric fruits (Lelievre *et al.*, 1997).

Sugars and organic acids are important elements determining the organoleptic features of any fruit (Mikulic *et al.*, 2012). The experimental results show that the

concentration of individual and total sugars in 'Fuji' did not vary much between 75 and 120 days cold stored fruit which is consistent with some previous reports (Sun *et al.*, 2000; Mikulic *et al.*, 2009a) claiming very minor changes in sugar levels of apple fruit during cold storage. Itai and Tanahashi (2008) have reported prevention of sugar loss in pears by 1-MCP application which is consistent with our results exhibiting positive effect of ethylene antagonists on stabilizing the sugar concentration in 'Fuji' fruit. Sugar levels in fruit change by the expression of various genes, which are responsible for sugar biosynthesis and degradation (Tanase and Yamaki, 2000). A reduction in the gene expression of sucrose degrading enzyme (acid invertase) in Japanese pear (*Pyrus pyrifolia* Nakai) has been reported with the application of 1-MCP (Itai and Tanahashi, 2008) which is probably responsible for generally more sugar content by ethylene antagonist in this study. Comparatively, more sucrose concentration in 'Fuji' after 120 days CS could be due to starch degradation, concomitant with climacteric stage of apples. A considerable increase in fructose content of 'Cripps Pink' was observed after long term storage of 120 days which is supported by earlier study of Veberic *et al.* (2010) on 'Jonagold' and 'Golden Delicious' apples. A robust decline in glucose level of 'Cripps Pink' is contrary to the reports of Ackermann *et al.* (1992) and Roth *et al.* (2007) indicating an increase in glucose during storage of 'Elstar' and 'Jonagold' apples respectively. In short, interconversion of sugars occurs during apple storage which depends highly on cultivars, chemical treatments and storage conditions.

Organic acids are substrates for fruit respiration with malic acid being the leading one in apple as it constitutes over 90 % of total organic acids. Interestingly, in both 'Fuji' and 'Cripps Pink' very minor changes in the levels of individual and total organic acids were observed with control and ethylene antagonists' treatment after 75 and 120 days cold storage. It seems that reduced respiration rate by ethylene antagonists helped to maintain the organic acid levels while in control fruit perhaps sugars have been utilized as a primary substrate resulting in stabilized organic acid content. An equivocal effect of ethylene antagonists on sugars and organic acids content of tested cultivars confirms that suppression of ethylene does not affect accumulation of these compounds (Pech *et al.*, 2012).

A decline in ascorbic acid content was observed in both cultivars after 75 and 120 days of CS to varying degrees with different ethylene antagonists except 1-MCP treatment which resulted in sustained concentration of ascorbic acid followed by (S)-(-)-

limonene treatment. Reduction in ascorbic acid levels of apples during storage is a common phenomenon as it is used in a defensive mechanism against free radicals during oxidative stress of stored fruit (Kevers *et al.*, 2011).

Cold storage intervals showed inconsistent effects on total phenolics in 'Fuji' and 'Cripps Pink' as data exhibited an increasing and decreasing trend simultaneously after CS periods (75 and 120 days) depending on the ethylene antagonist treatment. Similarly, previous studies on apple found a decline in phenolics during storage (Piretti *et al.*, 1994); while others have reported an increase (Burda *et al.*, 1990; Napolitano *et al.*, 2004) or no change with storage (Golding *et al.*, 2001). Among treatments, 1-MCP, 1-HCP and (*S*)-(-)-limonene maintained relatively higher levels of total phenolics in comparison to TCA and control.

Antioxidant capacity of apple is highly important for human nutrition as well as to maintain the fruit integrity during postharvest storage (Felicetti and Mattheis, 2010). 1-MCP and TCA fumigation treatments resulted in comparatively higher antioxidant levels in 'Fuji' and 'Cripps Pink' cultivars. It can be referred to a delay in mRNA transcripts of ethylene receptors such as ETR1, ERS1 and ERS2 by 1-MCP application (Tatsuki *et al.*, 2009) which may reduce the depletion of antioxidants due to the damaging effects of ethylene. Moreover, increased antioxidant capacity by TCA is justified by previous research on sweet pepper (Xing *et al.*, 2011) and melon (Carvalho *et al.*, 2016) demonstrating that TCA is able to maintain ascorbic acid and phenolic content of treated fruit, both of which are major markers of antioxidant concentration (Miller *et al.*, 1995). Positive impact of TCA could also be referred to its potential to scavenge free radicals (Carvalho *et al.*, 2016).

In conclusion, the fumigation of 1-MCP resulted in significantly paramount outcomes regarding ethylene and respiration rates, climacteric onset, fruit quality and biochemical constituents in 'Fuji' and 'Cripps Pink' after various cold storage periods. However, results indicate that 1-HCP, (*S*)-(-)-limonene and TCA have considerable potential to extend storage life of apple while simultaneously maintaining their quality attributes. Therefore, these compounds can be exploited commercially by the apple industry as productive alternatives to 1-MCP to maintain storage life and fruit quality.

## CHAPTER 5

### Effect of Ethylene Antagonists on CA Storage of Apple cv. 'Fuji' and 'Cripps Pink'

#### Abstract

Controlled atmosphere (CA) storage is commonly practised to extend the storage life and maintain quality of apple fruit. Ethylene antagonists overcome the adverse effects of ethylene during CA storage and consequently prolong the storage life and maintain fruit quality. The aim of the present investigation was to evaluate the efficacy of different ethylene antagonists i.e., 1-methylcyclopropene (1-MCP), 1-hexylcyclopropene (1-HCP), (*S*)-(-)-limonene and *trans*-cinnamaldehyde (TCA) on ethylene production, storage life and fruit quality in CA stored fruit of 'Fuji' and 'Cripps Pink' cultivars. Apple fruit were fumigated with 1-MCP (740 ppb), 1-HCP (1  $\mu$ M), (*S*)-(-)-limonene (1  $\mu$ M) and TCA (1  $\mu$ M) in sealed plastic containers for 24 hours. Untreated fruit served as a control. Following the fumigation treatments, the fruit were stored in CA comprising of 2 % O<sub>2</sub> and 1 % CO<sub>2</sub> at 1 °C for 6 and 8 months. After CA storage, rate of ethylene production and respiration were recorded for 10 days at simulated shelf conditions. Fruit quality parameters such as titratable acidity (TA), soluble solids concentration (SSC), SSC/TA ratio, colour, firmness and levels of ascorbic acid, total phenolics, total antioxidants, individual and total sugars and organic acids were determined on 10<sup>th</sup> day of shelf conditions. The rate of ethylene and CO<sub>2</sub> production was reduced to varying levels with fumigation treatments of all ethylene antagonists as compared to the control. 1-MCP and 1-HCP fumigation treatments delayed the onset of climacteric ethylene peaks by 5 - 6 days in 'Cripps Pink' fruit after 6 and 8 months of CA storage. In 1-MCP and 1-HCP treated 'Fuji' fruit, respiratory climacteric peaks were delayed for 1.7 and 1.3 days respectively after 6 months CA storage as compared to all other treatments and control. Losses in SSC, TA, SSC/TA ratio, fruit firmness and peel colour were reduced with all the fumigation treatments as compared to the control. Nonetheless, the intensity of colour (C\*) in 'Fuji' after 6 months CA storage was higher in control fruit as compared to all other treatments. 1-HCP, (*S*)-(-)-limonene and TCA maintained sugar levels, ascorbic acid, total phenolics and antioxidant levels in both apple cultivars. Conversely, organic acids were not significantly affected by ethylene antagonist treatments. 1-MCP had the more

pronounced effects on all quality parameters of tested cultivars in general, however, the effect of other ethylene antagonists varied with CA storage periods and cultivars. Although 1-MCP was found to be highly effective, however, positive effect of 1-HCP, (*S*)-(-)-limonene and TCA fumigation on ‘Fuji’ and ‘Cripps Pink’ fruit suggests that these compounds can be also used as an alternative to 1-MCP in the apple industry to extend CA storage life and maintain apple fruit quality.

### 5.1. Introduction

Apple is a popular fruit worldwide being a rich source of health benefiting compounds like polyphenols, antioxidants, dietary fibre, vitamins and minerals and their consumption can help to prevent pulmonary disorders, cancer, asthma and diabetes (Hyson, 2011). There are 7500 cultivars of apple which are grown worldwide in temperate and subtropical growing regions (Bapat *et al.*, 2010).

The apple industry of the world is facing intense competition in terms of supplying high quality fruit, which are treated with only GRAS (Generally Recognised as Safe) chemicals to prevent hazardous impact on human health and environment. Hence, dynamic pre and postharvest strategies are required by the commercial apple industry to protect domestic and international markets by providing quality produce (Harker *et al.*, 2003). Fruit quality is a composite of sensory and nutritional parameters which deteriorates through various phases of the supply chain (Martin-Diana *et al.*, 2007). Apple is a climacteric fruit and its quality starts to deteriorate immediately after harvesting due to autocatalytic ethylene biosynthesis during fruit ripening (Dandekar *et al.*, 2004). Ripening is a complex and irreversible process which is affected by genotype, environment, biotic and abiotic stress factors (Bapat *et al.*, 2010). Various plant growth regulators are involved in the fruit ripening process, but ethylene plays a key role. Ethylene binds to specific receptor sites to generate signals through a cascade i.e. CTR, EIN2, EIN3 to ERF (ethylene response factors) which ultimately express ripening related genes (Yang *et al.*, 2013).

Fresh horticulture commodities face postharvest losses of quality and quantity throughout the supply chain from the production site to consumers. According to the Food and Agriculture Organisation estimates, global food losses account for approximately 1.3 billion tonnes per annum (Lipinski *et al.*, 2013). Hence, operative measures are required to minimise the qualitative and quantitative postharvest losses

as we face the challenge of providing food security to about 9.1 billion population of the world by 2050 (Gogo *et al.*, 2017). Different types of growth regulators can regulate the effects of ethylene in plants including inhibitors of ethylene biosynthesis and inhibitors of ethylene action. The compounds which inhibit the action of ethylene in plants are known as ethylene antagonists and hold the promise to retard the fruit ripening processes and associated quality losses (Mir *et al.*, 2001). Hence, use of ethylene antagonists offers a promising approach to minimize postharvest losses of fresh horticultural commodities (Tucker *et al.*, 2017).

Moreover, commercial apple production and year round marketing necessitates optimum storage conditions in addition to reduced exposure to ethylene during storage/transportation and inhibition of ethylene action (Watkins, 2002) for prolonged storability, absence of diseases and physiological disorders, desired firmness levels, appealing aroma, good shape and colour. Therefore, efficient storage regimes are vital for the apple industry to satisfy the consumer's expected levels of fruit quality (Bapat *et al.*, 2010).

CA storage is a prevalent commercial practice which refers to the storage of fruits/vegetables in elevated carbon dioxide (CO<sub>2</sub>; above 1 %) and low oxygen (O<sub>2</sub>; less than 8 %) levels to extend the storage life and maintain produce quality (Kadafi, 1980; Gwanpua *et al.*, 2012). CA storage has been extensively used for long term apple availability in markets and it helps to meet the strict export standards better than regular air storage. Moreover, it facilitates to exploit the new markets around the globe by extending the green life i.e., time between harvesting and climacteric ripening (HAL, 2007). However, CA storage is linked to various physiological disorders in apples due to high CO<sub>2</sub> or low O<sub>2</sub> injury such as core browning, cortex browning or water core development (Argenta *et al.*, 2000). Both 'Fuji' and 'Cripps Pink' are CO<sub>2</sub> sensitive cultivars and demand optimum CA regimes with effective treatments prior to storage (de Castro *et al.*, 2007; Park *et al.*, 2011; Thewes *et al.*, 2017). Previous studies reveal that there is a possibility to overcome these issues by the exogenous application of ethylene antagonists in combination with a precise control of storage atmosphere (Fan *et al.*, 2005; Singh *et al.*, 2009; Argenta *et al.*, 2016).

Use of ethylene antagonists like 1-MCP have shown promise in long term CA storage of apples pertaining to quality retention and inhibition of certain disorders like

superficial scald and core flush by inhibiting ethylene action (Rupasinghe *et al.*, 2000; Zanella *et al.*, 2004; Park *et al.*, 2011). Some outstanding benefits of using 1-MCP during CA storage include inhibitory effects on the loss of firmness, fruit acidity, soluble solids, and reduced flesh browning in some apple cultivars (Fan *et al.*, 2005; Mattheis, 2008). Nonetheless, 1-MCP treated apple fruit after CA storage have shown some serious issues which compromise the fruit quality (Watkins and Nock, 2004 and 2012). Incidence of CO<sub>2</sub> injury and flesh browning in some apple varieties increases with fumigation of 1-MCP which varies with time interval between 1-MCP treatment and CA storage but still the treated fruit remain susceptible to these disorders (Jung *et al.*, 2009; Watkins and Nock, 2012). Moreover, CA storage reduces the production of volatiles which contribute to the characteristic apple aroma and 1-MCP application even further reduces the synthesis of volatile compounds (Lee *et al.*, 2012) affecting consumer acceptance.

This research study aimed to assess the responses of ‘Fuji’ and ‘Cripps Pink’ cultivars towards novel ethylene antagonists as an alternative to 1-MCP in CA conditions. For this purpose, the fruit were fumigated with 1-HCP, (S)-(-)-limonene, TCA and compared to 1-MCP as a standard. 1-HCP is structurally like 1-MCP and was shown to be an effective ethylene antagonist in previous studies (Kebenei *et al.*, 2003; Serek *et al.*, 2007; Khan *et al.*, 2016; Abdalghani, 2017), but no reports have been published on its effect on apples. (S)-(-)-limonene and TCA are found in essential oils and have been used as antimicrobial agents to prevent postharvest spoilage of fresh produce (Solgi and Ghorbanpour, 2014; Carvalho *et al.*, 2016). Moreover, (S)-(-)-limonene and TCA have been reported to inhibit ethylene induced flower abscission in waxflower (*Chamelaucium spp.*) by Abdalghani (2017), which exhibits potential of these compounds as ethylene antagonists. However, the efficacy of 1-HCP, (S)-(-)-limonene and TCA fumigation on climacteric ethylene production, storage life and quality of CA stored apple fruit warrants investigation.

Therefore, the present research investigation aimed at evaluating the effects of 1-MCP, 1-HCP, (S)-(-)-limonene and TCA fumigation in combination with CA storage on climacteric ethylene production, respiration rate, their corresponding climacteric peaks, storage life and quality of ‘Fuji’ and ‘Cripps Pink’ apple fruit.

### 5.2. Materials and Methods

### 5.2.1. Experiments

Two independent experiments were conducted to assess the efficiency of some ethylene antagonists on modulation of ethylene production, CA storage life and quality of 'Fuji' and 'Cripps Pink' apple fruit. Details of these experiments are given below.

#### 5.2.1.1. Experiment 1: Effect of ethylene antagonists on CA storage life and fruit quality of apple cv. 'Fuji'.

In this experiment, 'Fuji' apple fruit were fumigated with 1-MCP (740 ppb), 1-HCP (1  $\mu$ M), (*S*)-(-)-limonene (1  $\mu$ M) and TCA (1  $\mu$ M) compounds as ethylene antagonists for 24 hours in sealed plastic drums. Untreated fruit were kept as a control. The experiment was laid out by following a two-factor factorial (ethylene antagonists  $\times$  CA storage periods) completely randomized design (CRD) with three replications each having 20 fruit.

Following the fumigation treatment, the treated and control 'Fuji' fruit were stored in CA rooms comprising of 2 % O<sub>2</sub> and 1 % CO<sub>2</sub> at 1 °C and 95 % R.H. for a period of 6 months. After CA storage, fruit were moved to simulated shelf conditions (21  $\pm$  1°C) and ethylene production and respiration rate were recorded daily until post-climacteric stage. Quality parameters *viz.* firmness, fruit peel colour, TA, SSC, SSC/TA ratio, individual and total sugars, organic acids, ascorbic acid, total phenolics and total antioxidants were assayed on 10<sup>th</sup> day of simulated shelf conditions.

#### 5.2.1.2. Experiment 2: Effect of ethylene antagonists on CA storage life and fruit quality of apple cv. 'Cripps Pink'.

In this experiment, 'Cripps Pink' apple fruit were also fumigated with 1-MCP (740 ppb), 1-HCP (1  $\mu$ M), (*S*)-(-)-limonene (1  $\mu$ M) and TCA (1  $\mu$ M) ethylene antagonists. Experimental design was two-factor factorial (ethylene antagonists  $\times$  CA storage periods) CRD with three replications and 20 fruit in each replication. After fumigation with ethylene antagonists, 'Cripps Pink' fruit were stored in CA rooms (2 % O<sub>2</sub> and 1 % CO<sub>2</sub> at 1 °C and 95 % R.H.) for 6 and 8 months. Following CA storage periods, observations on the rate of ethylene production, respiration rate and various fruit quality parameters for 'Cripps Pink' were recorded as mentioned in experiment 1.

### **5.2.2. Fruit**

Apple cultivars of economic significance *viz.* ‘Fuji’ and ‘Cripps Pink’ at harvest maturity were obtained from a commercial orchard located in Manjimup (latitude 34°14’ South and longitude 116° 8’ East), Western Australia. Maturity of apple fruit was assessed by using starch pattern index as detailed in Chapter 3, Section 3.3.1. Uniform sized fruit free from any symptoms of disease, nutritional deficiency and mechanical damage were selected randomly from all parts of the tree canopy.

### **5.2.3. Experimental Site, Fumigation Treatments with Ethylene Antagonists and Storage Conditions**

Experiments were conducted in the Horticulture Research Laboratory, School of Molecular and Life Sciences, Bentley Campus, Curtin University, Perth, WA. Ethylene antagonists namely 1-MCP (740 ppb), 1-HCP (1  $\mu$ M), (*S*)-(-)-limonene (1  $\mu$ M) and TCA (1  $\mu$ M) were used for the fumigation of apple fruit. Detailed procedures for the fumigation treatment of ethylene antagonists and sources of chemicals are described in Chapter 3, Section 3.4. Following the treatment, the treated and control fruit were stored in CA rooms comprising of 2 % O<sub>2</sub> and 1 % CO<sub>2</sub> at 1°C and 95 % R.H. for a period of 6 and 8 months.

### **5.2.4. Estimation of Ethylene Production and Respiration Rate**

Following the CA storage periods, ethylene production rate from apple fruit was determined by using a Sensor Sense (ETD-300; Nijmegen, The Netherlands) and was expressed as pmol kg<sup>-1</sup> s<sup>-1</sup>. Respiration rate was estimated from uniform sized fruit of each experimental unit by infrared gas analyser (Servomex Series 1400, Sussex, UK) and expressed as  $\mu$ mol kg<sup>-1</sup> s<sup>-1</sup>. Determination procedures and essentials for the estimation of ethylene and respiration rates are mentioned in Chapter 3, Section 3.7.1 and 3.7.2.

### **5.2.5. Fruit Quality Parameters**

Skin colour was recorded by using a ColorFlex 45°/0° Spectrophotometer (Hunter Associates Inc., Reston, VA, USA) from two sides of apple fruit as L\*, a\*, b\*. Hue angle (h°) and Chroma (C\*) were calculated according to McGuire (1992) as described in Chapter 3, Section, 3.7.5.

Fruit firmness was determined by using a texture analyser (TA Plus, Lloyd's Instruments, Hampshire, UK) equipped with 11.1 mm diameter plunger on opposite dimensions of fruit in Newtons (N) as mentioned in Chapter 3, Section 3.7.4.

TA was assessed by titration method whereby 10 ml apple juice was extracted from 20 fruit in each replication and diluted with 20 ml distilled water then 5 ml aliquot was titrated with 0.1 N NaOH using phenolphthalein as an indicator. TA was presented as malic acid equivalents (%). Furthermore, SSC was assayed by a digital refractometer (ATAGO™ Digital Palette Refractometer, PR-101, Tokyo, Japan) from apple juice and expressed as a percentage (%). To estimate SSC/TA ratio, SSC values were divided by corresponding TA values. Detailed procedures for the estimation of TA, SSC and SSC/TA ratio have been given in Chapter 3, Section 3.7.3.

### 5.2.6. Determination of Individual and Total Sugars and Organic Acids

Apple pulp (5 g) was homogenized with 45 ml Milli-Q water and centrifuged at 10,000 *g* for 15 min at 4 °C. Supernatant was filtered by Nylon syringe filters (0.2 µm) into 1 ml HPLC glass vials. The individual and total sugars and organic acids were identified and quantified by using a HPLC system (Waters, 717plus, Milford Corp, MA, USA) fitted with a refractive index (RI) detector and UV-absorbance detector for sugars and organic acids respectively. The concentrations of individual sugars and organic acids were expressed as g kg<sup>-1</sup> (Khan *et al.*, 2016). Analytical method for HPLC is comprehensively described in Chapter 3, Section 3.7.9.

### 5.2.7. Estimation of Ascorbic Acid, Total Phenolics and Antioxidant Capacity

Apple pulp (5 g) with 25 ml of 6 % metaphosphoric acid solution was ground with homogenizer (Heidolph DIAX 900, Sydney, Australia) and centrifuged at 5000 *g* for 20 min. An aliquot (400 µl) from supernatant, diluted with 200 µl of metaphosphoric acid solution (3 %), 1400 µl d.H<sub>2</sub>O and 200 µl Folin's reagent (5 times diluted with d.H<sub>2</sub>O) was used to check the absorbance of samples at 760 nm by a UV spectrophotometer (Model 6405, Dunmow, Essex, UK). The concentration of ascorbic acid in the pulp was calculated using standard curve of L-ascorbic acid (Wan Zaliha, 2009). The concentration of ascorbic acid was expressed as mg Kg<sup>-1</sup>.

Folin–Ciocalteu (FC) assay was used to determine total phenolics by grinding 10 g apple pulp with 90 ml of 70 % acetone and mixing well for 1 hour in orbital

shaker at 170 rpm. Grinded samples were centrifuged at 2500 g for 15 min with subsequent dilution of 0.5 ml supernatant with 3 ml d.H<sub>2</sub>O and 0.25 ml FC-reagent. The samples were incubated at 37 °C for 30 min with further addition of 0.75 ml sodium carbonate solution (20 %) and 0.95 ml d.H<sub>2</sub>O and absorbance was recorded using a UV spectrophotometer (Model 6405, Dunmow, Essex, UK) at 765 nm. Gallic acid standard curve was used for the calculation of total phenolics in apple samples and expressed as mg Kg<sup>-1</sup> (Henriquez *et al.*, 2010).

To determine total antioxidants in apple pulp and peel, DPPH assay was used as described previously by Brand-Williams *et al.* (1995). The extraction buffer (2 mM NaF solution in 200 ml d.H<sub>2</sub>O and 800 ml methanol) (10 ml) was used to mash 15 g apple pulp and 0.5 g peel. After centrifugation for 20 min at 5000 g, various volumes of supernatant depending upon treatments and replications, mixed with 950 µl of DPPH solution (2,2-diphenyl-1-picrylhydrazyl) were subjected to UV-spectrophotometry (Model 6405, Dunmow, Essex, UK) to check absorbance at 515 nm after 15 min, while absorbance of DPPH solution was set at 1.1. Standard curve of Trolox was used for the determination of total antioxidants which were expressed as mM Trolox Kg<sup>-1</sup>.

### 5.2.8. Statistical Analysis

The data were subjected to two-way analysis of variance (ANOVA) using GenStat 14.1. Means were separated by least significance difference (LSD) test at  $P \leq 0.05$ .

## 5.3. Results

### 5.3.1. Ethylene Production and Respiration Rate

Climacteric ethylene production rate and respiration rate peaks in ‘Fuji’ and ‘Cripps Pink’ apple were reduced and delayed by ethylene antagonist treatments after 6 and 8 months CA storage (Table 5.1 - 5.4).

In ‘Cripps Pink’ apple fruit, ethylene antagonists significantly suppressed and delayed the onset of ethylene climacteric peaks following CA storage periods (Table 5.1). Mean days to the onset of ethylene climacteric were significantly delayed by 1-MCP and 1-HCP treatments (6.0 and 5.5 days respectively) in comparison to control

and all other treatments. Six months CA storage significantly delayed mean days to ethylene climacteric peak by 5.93 days than 8 months CA storage (3.20). Interaction of ethylene antagonists and CA storage was significant for the onset of climacteric ethylene peak in ‘Cripps Pink’. 1-MCP and 1-HCP treatments significantly delayed the onset of ethylene climacteric by 6.0 days after 6 months CA storage of ‘Cripps Pink’; while after 8 months CA storage, 1-MCP and 1-HCP fumigation treatments led to the delay of 6.0 and 5.0 days respectively as compared to control and other ethylene antagonists (Table 5.1).

Mean rate of ethylene production peak in ‘Cripps Pink’ was significantly lower with 1-MCP treatment ( $200.7 \text{ pmol Kg}^{-1} \text{ s}^{-1}$ ) followed by (*S*)-(-)-limonene ( $874.5 \text{ pmol Kg}^{-1} \text{ s}^{-1}$ ) and TCA ( $904.2 \text{ pmol Kg}^{-1} \text{ s}^{-1}$ ) as compared to 1-HCP and control (Table 5.1). ‘Cripps Pink’ fruit after 8 months CA storage exhibited significantly reduced mean ethylene production ( $358.2 \text{ pmol Kg}^{-1} \text{ s}^{-1}$ ) than 6 months CA storage ( $1469.2 \text{ pmol Kg}^{-1} \text{ s}^{-1}$ ). Interaction of ethylene antagonists and CA storage periods was found to be significant for ethylene peak rate in ‘Cripps Pink’. 1-MCP treated fruit had significantly suppressed rate of ethylene production at climacteric peak after 6 months ( $354 \text{ pmol Kg}^{-1} \text{ s}^{-1}$ ) and 8 months CA storage ( $47.2 \text{ pmol Kg}^{-1} \text{ s}^{-1}$ ) in comparison to all other treatments and control (Table 5.1).

None of the fumigation treatments resulted in significant delay in onset of the climacteric ethylene peak as compared to the control in 6 months CA stored ‘Fuji’ fruit. However, all the treatments except TCA suppressed climacteric ethylene production rate at peak as compared to the control in 6 months CA stored ‘Fuji’ fruit (Table 5.2). 1-MCP was most effective followed by 1-HCP and (*S*)-(-)-limonene in reducing climacteric ethylene production at peak as compared to all other treatments and control in ‘Fuji’.

In ‘Cripps Pink’, fumigation treatments with TCA, 1-MCP and (*S*)-(-)-limonene significantly delayed (2.34, 1.84 and 1.5 days respectively) onset of climacteric respiration peaks as compared to the control and 1-HCP fumigation (Table 5.3). CA storage periods and their interaction with ethylene antagonists were found to be non-significant for the onset of respiratory climacteric peak in ‘Cripps Pink’.

Mean rates of respiratory peak in ‘Cripps Pink’ were significantly suppressed with different ethylene antagonist treatments (Table 5.3). 1-MCP greatly restrained

the peak rate of respiration ( $0.12 \mu\text{mol Kg}^{-1} \text{s}^{-1}$ ) followed by 1-HCP, (*S*)-(-)-limonene and TCA ( $0.17 - 0.24 \mu\text{mol Kg}^{-1} \text{s}^{-1}$ ) in comparison to control. Nonetheless, CA storage periods and their interaction with fumigation treatments did not show any significant impact on peak rate of respiration in 'Cripps Pink' (Table 5.3).

The onset of respiratory climacteric peak was delayed for 1.7 and 1.3 days with 1-MCP and 1-HCP fumigation treatments respectively in 'Fuji' after 6 months CA storage. However, the effect of ethylene antagonists on climacteric respiration rate was found to be non-significant in 6 months CA stored 'Fuji' fruit (Table 5.4).

Table 5.1. Effects of ethylene antagonists on peak rate of ethylene production and number of days to the onset of ethylene climacteric peak in apple cv. 'Cripps Pink' after CA storage.

Ethylene antagonists	Ethylene Climacteric					
	Peak Rate (Ethylene $\mu\text{mol kg}^{-1}\text{s}^{-1}$ )		Mean (T)	Onset (days)		Mean (T)
	6M-CA	8M-CA		6M-CA	8M-CA	
Control	2307.5 $\pm$ 80.4c	497.1 $\pm$ 12.5a	1402.3c	3.00 $\pm$ 0.24b	2.00 $\pm$ 0.0a	2.50a
1-MCP	354.2 $\pm$ 5.6a	47.2 $\pm$ 1.0a	200.7a	9.00 $\pm$ 0.0f	8.00 $\pm$ 0.0e	8.50c
1-HCP	2019.0 $\pm$ 209.4c	355.4 $\pm$ 2.6a	1187.2bc	9.00 $\pm$ 0.0f	7.00 $\pm$ 0.0d	8.00d
(S)-(-)-Lim	1324.0 $\pm$ 9.8b	424.9 $\pm$ 2.7a	874.5b	4.33 $\pm$ 0.14c	4.00 $\pm$ 0.0c	4.16bc
TCA	1342.0 $\pm$ 45.9b	466.5 $\pm$ 13.4a	904.2b	4.33 $\pm$ 0.27c	3.00 $\pm$ 0.0b	3.66b
Mean (SP)	1469.2b	358.2a		5.93b	3.20a	
LSD ( $P \leq 0.05$ )	T = 358.1	SP = 226.5	T $\times$ SP = 506.4	T = 0.63	SP = 0.49	T $\times$ SP = 0.89

6M-CA = storage in controlled atmosphere for 6 months, 8M-CA = storage in controlled atmosphere for 8 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu\text{M}$ ), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu\text{M}$ ), TCA = *trans*-cinnamaldehyde (1  $\mu\text{M}$ ), T = Treatment, SP = Storage period.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 5.2. Effects of ethylene antagonists on peak rate of ethylene production and number of days to the onset of ethylene climacteric peak in apple cv. 'Fuji' after CA storage.

Ethylene antagonists	Ethylene Climacteric	
	Fuji (6M-CA)	
	Peak Rate (Ethylene pmol kg <sup>-1</sup> s <sup>-1</sup> )	Onset (days)
Control	1076.5±55.5d	3.0±0.0
1-MCP	145.9±3.7a	7.0±0.2
1-HCP	630.5±2.8b	3.3±0.1
(S)-(-)-Lim	703.2±22.7bc	5.3±0.5
TCA	867.9±36.6cd	5.0±0.6
LSD ( $P \leq 0.05$ )	210.4	NS

6M-CA = storage in controlled atmosphere for 6 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 5.3. Effects of ethylene antagonists on peak rate of respiration (CO<sub>2</sub>) and number of days to the onset of respiratory climacteric peak in apple cv. 'Cripps Pink' after CA storage.

Ethylene antagonists	Respiratory Climacteric					
	Peak rates (CO <sub>2</sub> μmol kg <sup>-1</sup> s <sup>-1</sup> )		Mean (T)	Onset (days)		Mean (T)
	6M-CA	8M-CA		6M-CA	8M-CA	
Control	0.33±0.01	0.41±0.04	0.37c	4.33±0.14	5.3±0.14	4.83a
1-MCP	0.12±0.0	0.13±0.01	0.12a	7.00±0.0	6.3±0.54	6.67b
1-HCP	0.13±0.0	0.34±0.03	0.24b	5.33±0.36	6.3±0.14	5.83a
(S)-(-)-Lim	0.15±0.01	0.22±0.01	0.19ab	5.33±0.14	7.3±0.36	6.33b
TCA	0.14±0.0	0.20±0.01	0.17ab	7.00±0.0	7.3±0.36	7.17b
Mean (SP)	0.173	0.262		5.80	6.53	
LSD ( $P \leq 0.05$ )	T = 0.09	SP = NS	T × SP = NS	T = 1.37	SP = NS	T × SP = NS

6M-CA = storage in controlled atmosphere for 6 months, 8M-CA = storage in controlled atmosphere for 8 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 μM), (S)-(-)-Lim = (S)-(-)-limonene (1 μM), TCA = *trans*-cinnamaldehyde (1 μM), T = Treatment, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 5.4. Effects of ethylene antagonists on peak rate of respiration (CO<sub>2</sub>) and number of days to the onset of respiratory climacteric peak in apple cv. 'Fuji' after CA storage.

Ethylene antagonists	Respiratory Climacteric	
	Fuji (6M-CA)	
	Peak rates (CO <sub>2</sub> μmol kg <sup>-1</sup> s <sup>-1</sup> )	Onset (days)
Control	0.30±0.00	7.0±0.00a
1-MCP	0.28±0.02	8.7±0.14c
1-HCP	0.25±0.01	8.3±0.14bc
(S)-(-)-Lim	0.27±0.01	7.7±0.14ab
TCA	0.24±0.01	7.0±0.00a
LSD ( $P \leq 0.05$ )	NS	0.91

6M-CA = storage in controlled atmosphere for 6 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP, 1-hexylcyclopropene (1 μM), (S)-(-)-Lim = (S)-(-)-limonene (1 μM), TCA = *trans*-cinnamaldehyde (1 μM), NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

### **5.3.2. Fruit Quality Parameters**

Effect of ethylene antagonists on fruit quality i.e. skin colour, fruit firmness, TA, SSC and SSC/TA was found to be significant in ‘Fuji’ and ‘Cripps Pink’ apple cultivars. CA storage intervals also influenced these quality attributes in ‘Cripps Pink’ (Table 5.5 - 5.8).

#### **5.3.2.1. Fruit Colour**

Mean  $L^*$  and  $b^*$  values in ‘Cripps Pink’ fruit were significantly lowest with fumigation of 1-MCP (48.8 and 25.6), 1-HCP (50.5 and 26.2) and (*S*)-(-)-limonene treatments (49.2 and 25.8) in comparison to TCA and control (Table 5.5). Six months CA stored fruit exhibited significantly lower mean fruit lightness ( $L^*$ ) and yellowness ( $b^*$ ) (50.1 and 26.8 respectively) than those stored for 8 months (52.2 and 28.1 respectively). Interaction of fumigation treatments and CA storage was found to be significant for  $L^*$  and  $b^*$ . Fumigation of 1-MCP and (*S*)-(-)-limonene significantly reduced  $L^*$  (47.0 and 46.3 respectively) and  $b^*$  values (24.6 and 23.5 respectively) in 6 months CA stored ‘Cripps Pink’ fruit (Table 5.5).

Mean values for skin red blush ( $a^*$ ) were significantly higher in 1-HCP (36.8) and (*S*)-(-)-limonene (37.3) treated ‘Cripps Pink’ fruit as compared to all other treatments and control. Mean  $a^*$  value was significantly higher after 6 months CA storage (36.4) than 8 months (32.7). Ethylene antagonists and CA storage periods interacted significantly for  $a^*$  in ‘Cripps Pink’ with significantly more value in 1-HCP (38.8) and (*S*)-(-)-limonene (42.4) treated fruit (Table 5.5) as compared to control and other treatments.

Mean chroma ( $C^*$ ) in ‘Cripps Pink’ was significantly decreased with 1-MCP fumigation treatment (41.7) in comparison to control and all other treatments. Mean chroma was significantly higher in 6 months CA stored fruit (45.9) than 8 months (43.9). The interaction of CA storage periods and ethylene antagonist’s treatments was found to be significant with relatively reduced  $C^*$  values in 1-MCP treated ‘Cripps Pink’ fruit after 6 and 8 months of CA storage (41.3 and 42.0 respectively) (Table 5.5).

Mean hue angle ( $h^\circ$ ) was significantly declined with 1-HCP (34.7) and (*S*)-(-)-limonene (35.3) treatments in comparison to control and all other treatments. Six

months CA stored fruit showed significantly reduced  $h^\circ$  values (36.9) than 8 months (40.7). There was a significant interaction between CA storage periods and fumigation treatments which resulted in lower hue angle ( $h^\circ$ ) in (*S*)-(-)-limonene treated ‘Cripps Pink’ fruit (29.0) after 6 months CA storage (Table 5.5).

In ‘Fuji’ fruit, all the fumigation treatments reduced  $L^*$  value (49.4 - 51.6) compared to the control (52.5). Fruit red blush ( $a^*$ ) was significantly higher (17.5) with 1-HCP fumigation treatment in comparison to all other treatments and control. After long-term CA storage of ‘Fuji’ (6 months), fruit yellowness ( $b^*$ ) was significantly less with 1-HCP (27.5) and (*S*)-(-)-limonene (26.4) fumigation treatments as compared to control and other ethylene antagonists. Significantly increased chroma ( $C^*$ ) was observed in control ‘Fuji’ fruit (36.9), while hue angle ( $h^\circ$ ) was significantly reduced with 1-HCP fumigation treatment (55.8) in comparison to all other treatments and control (Table 5.6).

### 5.3.2.2. Fruit Firmness

Fumigation treatments with ethylene antagonists and CA storage resulted in reduced loss of fruit firmness in ‘Fuji’ and ‘Cripps Pink’ apple (Table 5.7 and 5.8). All the ethylene antagonist fumigation treatments resulted in significantly higher fruit firmness following CA storage as compared to the control. Mean fruit firmness in ‘Cripps Pink’ was found to be significantly higher with 1-MCP fumigation treatment (74.4 N) followed by 1-HCP, (*S*)-(-)-limonene and TCA treatments (70.9 - 72.1 N) in comparison to control (61.1N) (Table 5.7). The mean fruit firmness was higher in 6 months CA stored fruit (71.9 N) than 8 months (68.1 N). Significant interaction was observed between fumigation treatments and CA storage periods for ‘Cripps Pink’ fruit firmness. 1-MCP and 1-HCP treated ‘Cripps Pink’ fruit exhibited higher fruit firmness (77.3 and 74.7 N respectively) after 6 months CA storage as compared to all other treatments and control. After 8 months CA storage, significantly higher fruit firmness was recorded in 1-MCP, 1-HCP and (*S*)-(-)-limonene treated ‘Cripps Pink’ fruit (71.6, 69.5 and 70.6 N respectively) in comparison to TCA and control (Table 5.7).

All the ethylene antagonist fumigation treatments resulted in significantly higher fruit firmness in ‘Fuji’ after 6 months of CA storage (69.6 - 70.2 N) as compared to the control (62.9 N) (Table 5.8).

**5.3.2.3. TA, SSC and SSC/TA ratio**

All the ethylene antagonist fumigation treatments exhibited significantly higher mean TA in 'Cripps Pink' fruit after CA storage as compared to the control, but 1-MCP was most effective (Table 5.7). Interaction of CA storage periods and ethylene antagonists produced significantly higher TA in 1-MCP treated 'Cripps Pink' fruit (0.64 %) after 6 months of CA storage. Mean SSC was found to be significantly higher with 1-MCP (14.8 %) and 1-HCP (14.4 %) treatments in comparison to control and other fumigation treatments. Mean SSC was significantly more after 6 months CA storage (14.4 %) than 8 months (13.8 %). Interaction of ethylene antagonists and CA storage periods was significant for SSC percentage in 'Cripps Pink'. 1-MCP treatment resulted in significantly higher SSC (15.1 %) after 6 months of CA storage as compared to all other treatments and control. However, after 8 months CA storage, 1-HCP treatment produced an increased SSC (14.1 %) in 'Cripps Pink' followed by control (14.0 %) in comparison to other treatments. All fumigation treatments significantly reduced the mean SSC/TA ratio in 'Cripps Pink' (27.2 to 31.7) than control (37.1). CA storage periods and their interaction with fumigation treatments had non-significant effect on SSC/TA ratio of 'Cripps Pink' apple cultivar (Table 5.7).

In 'Fuji', significantly higher TA (0.34 %) was observed in 1-MCP treated fruit as compared to all other treatments and control after 6 months CA storage. Maximum SSC was recorded in 1-MCP treated 'Fuji' fruit (12.7 %) followed by (*S*)-(-)-limonene and control fruit (12.5 %), while significantly higher SSC/TA ratio was found in control 'Fuji' fruit in comparison to treatments subjected to ethylene antagonists (Table 5.8).

Table 5.5. Effect of ethylene antagonists on fruit colour parameters of apple cv. 'Cripps Pink' after CA storage.

Ethylene antagonists	Fruit Colour														
	L*		Mean	a*		Mean	b*		Mean	C*		Mean	h°		Mean
	6M-CA	8M-CA	(T)	6M-CA	8M-CA	(T)	6M-CA	8M-CA	(T)	6M-CA	8M-CA	(T)	6M-CA	8M-CA	(T)
Control	54.4±0.3de	53.1±0.1cd	53.7c	32.3±0.1ab	33.8±0.4bc	33.1ab	30.8±0.1e	28.7±0.3d	29.8b	45.8±0.0cd	45.1±0.1c	45.4b	44.3±0.2ef	40.8±0.6de	42.5c
1-MCP	47.0±0.1a	50.6±0.2b	48.8a	32.1±0.2ab	31.8±0.3ab	31.9a	24.6±0.1a	26.7±0.3bc	25.6a	41.3±0.1a	42.0±0.4a	41.7a	38.5±0.2cd	40.4±1.3de	39.5b
1-HCP	51.1±0.3bc	49.9±0.4b	50.5b	38.8±0.1e	34.8±0.2cd	36.8c	26.3±0.3b	26.1±0.3b	26.2a	47.1±0.2d	44.6±0.3bc	45.9b	34.3±0.2b	35.1±0.3bc	34.7a
(S)-(-)-Lim	46.3±0.2a	51.9±0.4bc	49.2ab	42.4±0.2f	32.2±0.3ab	37.3c	23.5±0.1a	28.0±0.3cd	25.8a	48.7±0.1e	43.5±0.1b	46.1b	29.0±0.2a	41.5±0.5de	35.3a
TCA	51.6±0.2bc	55.6±0.2e	53.6c	36.5±0.2d	30.7±0.2a	33.6b	28.9±0.2d	30.8±0.2e	29.9b	46.9±0.3d	44.3±0.1bc	45.6b	38.7±0.1cd	45.5±0.4f	42.1c
Mean (SP)	50.1a	52.2b		36.4b	32.7a		26.8a	28.1b		45.9b	43.9a		36.9a	40.7b	
LSD ( $P \leq 0.05$ )	T=1.37, SP=0.9, T×SP=1.9			T=1.3, SP=0.8, T×SP=1.8			T=1.0, SP=0.7, T×SP=1.5			T=1.0, SP=0.6, T×SP=1.4			T=2.5, SP=1.6, T×SP=3.6		

6M-CA = storage in controlled atmosphere for 6 months, 8M-CA = storage in controlled atmosphere for 8 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = Treatment, SP = Storage period. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 5.6. Effect of ethylene antagonists on fruit colour parameters of apple cv. 'Fuji' after CA storage.

Ethylene antagonists	Fruit Colour				
	Fuji (6M-CA)				
	L*	a*	b*	C*	h°
Control	52.5±0.1b	14.3±0.0a	29.9±0.3b	36.9±0.2c	64.3±0.0c
1-MCP	51.1±0.1ab	15.4±0.1b	29.2±0.1b	35.4±0.2b	63.5±0.4c
1-HCP	50.1±0.2ab	17.5±0.2c	27.5±0.1a	34.6±0.1b	55.8±0.0a
(S)-(-)-Lim	51.6±0.2ab	15.3±0.0b	26.4±0.2a	32.1±0.1a	60.3±0.0b
TCA	49.4±0.5a	13.6±0.1a	30.0±0.2b	34.4±0.2b	64.0±0.0c
LSD ( $P \leq 0.05$ )	2.28	0.83	1.57	0.97	1.45

6M-CA = storage in controlled atmosphere for 6 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M).  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 5.7. Effect of ethylene antagonists on TA, SSC, SSC/TA ratio and firmness of apple cv. 'Cripps Pink' after CA storage.

Ethylene antagonists	TA (%)		Mean (T)	SSC (%)		Mean (T)	SSC/TA ratio		Mean (T)	Firmness (N)		Mean (T)
	6M-CA	8M-CA		6M-CA	8M-CA		6M-CA	8M-CA		6M-CA	8M-CA	
Control	0.37±0.01a	0.39±0.01a	0.38a	13.5±0.02	14.0±0.02de	13.8a	37.6±0.9	36.6±1.4	37.1b	61.2±0.3a	61.0±0.5a	61.1a
1-MCP	0.64±0.01e	0.56±0.01d	0.60c	15.1±0.02	13.9±0.01d	14.5c	23.5±0.3	30.9±2.2	27.2a	77.3±0.1e	71.6±0.4cd	74.4c
1-HCP	0.44±0.01abc	0.47±0.01b	0.46b	14.7±0.02	14.1±0.01e	14.4c	33.4±0.7	30.0±0.3	31.7a	74.7±0.9de	69.5±0.1bc	72.1b
(S)-(-)-Lim	0.4±0.01abc	0.47±0.02b	0.45b	14.6±0.02	13.3±0.01a	13.9b	34.1±0.5	28.9±1.1	31.5a	71.3±0.2c	70.6±0.4bc	70.9b
TCA	0.48±0.01c	0.40±0.0ab	0.44b	14.1±0.02	13.7±0.02c	13.9b	29.4±0.5	34.1±0.1	31.7a	75.2±0.5e	67.6±0.6b	71.4b
Mean (SP)	0.47	0.46		14.4b	13.8a		31.6	32.1		71.9b	68.1a	
LSD ( $P \leq 0.05$ )	T = 0.05, SP = NS, T×SP = 0.07			T = 0.11, SP = 0.07, T×SP = 0.16			T = 4.9, SP = NS, T×SP = NS			T = 2.27, SP = 1.44, T×SP = 3.22		

6M-CA = storage in controlled atmosphere for 6 months, 8M-CA = storage in controlled atmosphere for 8 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M). T = Treatment, SP = Storage period. NS = Non-significant.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 5.8. Effect of ethylene antagonists on TA, SSC, SSC/TA ratio and firmness of apple cv. 'Fuji' after CA storage.

Ethylene antagonists	Fuji (6M-CA)			
	TA (%)	SSC (%)	SSC/TA ratio	Firmness (N)
Control	0.17±0.01a	12.5±0.02ab	72.5±2.01c	62.9±0.26a
1-MCP	0.34±0.01c	12.7±0.02b	38.0±0.60a	70.2±0.35b
1-HCP	0.21±0.01ab	12.2±0.04a	57.4±1.23b	68.6±0.24b
(S)-(-)-Lim	0.23±0.01b	12.5±0.07ab	55.5±1.68b	69.6±0.32b
TCA	0.25±0.01b	12.2±0.02a	48.2±1.06ab	69.9±0.27b
LSD ( $P \leq 0.05$ )	0.05	0.33	11.7	2.42

6M-CA = storage in controlled atmosphere for 6 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M),  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

#### **5.3.2.4. Individual and Total Sugars**

Data for individual and total sugars in 'Fuji' and 'Cripps Pink' varied significantly by ethylene antagonist treatments and CA storage (Table 5.9 and 5.10). Mean concentrations of glucose, fructose and total sugars in 'Cripps Pink' were significantly higher in 1-MCP fumigated fruit (11.9, 78.8 and 125.6 g Kg<sup>-1</sup> respectively) as compared to all other treatments and control. However, mean concentration of sucrose was found to be significantly higher in control fruit of 'Cripps Pink' (41.3 g Kg<sup>-1</sup>) in comparison to all fumigation treatments. CA storage period means indicate significantly high concentrations of sucrose and glucose after 8 months CA storage (38.2 and 9.8 g Kg<sup>-1</sup> respectively), while mean concentrations of fructose and total sugars were significantly higher (80.4 and 122.2 g Kg<sup>-1</sup> respectively) after 6 months CA storage of 'Cripps Pink' (Table 5.9).

Interaction of CA storage periods (6 and 8 months) and fumigation treatments with ethylene antagonists was significant for individual and total sugars of 'Cripps Pink' except sucrose. 1-MCP treatment led to significantly higher glucose concentration (13.7 g Kg<sup>-1</sup>) after 8 months of CA storage in comparison to all other treatments and control. Concentration of fructose was quantified to be significantly higher by HPLC in 1-MCP and TCA treated 'Cripps Pink' fruit (85.6 and 84.9 g Kg<sup>-1</sup>) after 6 months CA storage, when compared to all other treatments and control. Similarly, 1-MCP and TCA treatments also resulted in significantly increased concentration of total sugars after 6 months CA storage of 'Cripps Pink' (127.3 and 128.9 g Kg<sup>-1</sup>) than control and all other treatments. After 8 months CA storage, concentrations of total sugars were relatively greater in 1-MCP treated 'Cripps Pink' fruit (123.8 g Kg<sup>-1</sup>) in comparison to all other ethylene antagonists and control (Table 5.9).

'Fuji' apple showed a significantly higher concentration of glucose (27.7 g Kg<sup>-1</sup>), fructose (65.5 g Kg<sup>-1</sup>) and total sugars (122.0 g Kg<sup>-1</sup>) when fumigated with (*S*)-(-)-limonene in comparison to all other treatments and control after 6 months CA storage. Following (*S*)-(-)-limonene, 1-HCP treatment resulted in comparatively higher glucose concentration in 'Fuji' fruit while relatively higher concentration of fructose and total sugars was recorded in TCA treated fruit and control as compared to all other

treatments. The effect of ethylene antagonists on sucrose concentration of 'Fuji' was not significant (Table 5.10).

Table 5.9. Effect of ethylene antagonists on individual and total sugars of apple cv. 'Cripps Pink' after CA storage.

Ethylene antagonists	Sugars (g kg <sup>-1</sup> )											
	Sucrose		Mean (T)	Glucose		Mean (T)	Fructose		Mean (T)	Total Sugars		Mean (T)
	6M-CA	8M-CA		6M-CA	8M-CA		6M-CA	8M-CA		6M-CA	8M-CA	
Control	38.4±0.1	44.2±0.4	41.3b	5.11±0.0a	8.56±0.0d	6.8a	73.3±0.2d	59.8±0.0a	66.5a	116.8±0.2bc	112.5±0.5b	114.6a
1-MCP	31.8±0.1	37.9±0.4	34.9a	9.97±0.0f	13.78±0.0i	11.9e	85.6±0.3f	72.0±0.1c	78.8d	127.3±0.3e	123.8±0.4de	125.6b
1-HCP	27.3±0.1	36.9±1.4	32.1a	10.26±0.0g	11.09±0.0h	10.7d	79.3±0.1e	66.5±0.1b	72.9c	116.8±0.2bc	114.5±1.3b	115.6a
(S)-(-)-Lim	33.9±0.0	37.1±1.6	35.5a	8.19±0.0c	6.44±0.0b	7.3b	79.2±0.1e	60.1±0.1a	69.6b	121.2±0.1cd	103.6±1.5a	112.4a
TCA	35.3±0.0	35.1±0.9	35.2a	8.66±0.0d	9.31±0.0e	8.9c	84.9±0.2f	59.4±0.1a	72.1c	128.9±0.2e	103.7±1.0a	116.3a
Mean (SP)	33.3a	38.23b		8.5a	9.8b		80.4b	63.5a		122.2b	111.6a	
LSD ( $P \leq 0.05$ )	T = 3.9, SP = 2.5, T×SP = NS			T = 0.08, SP = 0.1, T×SP = 1.1			T = 0.7, SP = 0.5, T×SP = 1.0			T = 3.9, SP = 2.5, T×SP = 5.5		

6M-CA = storage in controlled atmosphere for 6 months, 8M-CA = storage in controlled atmosphere for 8 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM). T = Treatment, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 5.10. Effect of ethylene antagonists on individual and total sugars of apple cv. 'Fuji' after CA storage.

Ethylene antagonists	Sugars (g kg <sup>-1</sup> ) Fuji (6M-CA)			
	Sucrose	Glucose	Fructose	Total Sugars
Control	26.6±1.32	25.0±0.01b	57.8±0.18bc	109.3±1.42b
1-MCP	21.3±1.40	25.7±0.04c	48.9±0.19a	95.9±1.46a
1-HCP	26.1±0.06	26.9±0.01d	56.1±0.07b	109.1±0.11b
(S)-(-)-Lim	28.8±0.02	27.7±0.03e	65.5±0.21d	122.0±0.23c
TCA	28.8±0.02	24.1±0.03a	58.7±0.30c	111.5±0.28b
LSD ( $P \leq 0.05$ )	NS	0.21	1.75	7.91

6M-CA = storage in controlled atmosphere for 6 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

### 5.3.2.5. Individual and Total Organic Acids

Individual organic acids, which were identified and quantified in ‘Fuji’ apple are; malic acid, succinic acid and fumaric acid while in ‘Cripps Pink’, citric acid was also identified in addition to the aforementioned organic acids. Fumigation treatments with ethylene antagonists and CA storage did not affect the individual and total organic acids significantly in ‘Fuji’ and ‘Cripps Pink’ apple with few exceptions which are interpreted here (Table 5.11 and 5.12).

Mean concentration of malic acid was relatively higher in 1-HCP treated ‘Cripps Pink’ fruit ( $6.64 \text{ g Kg}^{-1}$ ) as compared to other ethylene antagonists and control. However, it was parallel to mean malic acid concentration in 1-MCP and (*S*)-(-)-limonene treatments ( $5.92$  and  $5.91 \text{ g Kg}^{-1}$  respectively). Mean concentration of succinic acid was considerably low in 1-HCP fumigation treatment ( $0.09 \text{ g Kg}^{-1}$ ) in comparison to all other treatments and control. All fumigation treatments resulted in comparatively higher mean total organic acids in ‘Cripps Pink’ ( $12.3$ - $15.2 \text{ g Kg}^{-1}$ ) than control (Table 5.11).

Interaction of CA storage periods and ethylene antagonist treatments was found to be significant for malic acid and succinic acid in ‘Cripps Pink’. After 6 months CA storage, 1-HCP treatment produced significantly higher malic acid ( $7.93 \text{ g Kg}^{-1}$ ) while after 8 months CA storage, 1-MCP and (*S*)-(-)-limonene treated ‘Cripps Pink’ fruit showed relatively high concentration of malic acid ( $6.44$  and  $6.17 \text{ g Kg}^{-1}$ ) than TCA and control. Succinic acid concentration was high in control ( $0.43 \text{ g Kg}^{-1}$ ) and TCA ( $0.25 \text{ g Kg}^{-1}$ ) after 6 months CA storage while after 8 months succinic acid was significantly higher in (*S*)-(-)-limonene treatment ( $0.54 \text{ g Kg}^{-1}$ ) followed by TCA ( $0.33 \text{ g Kg}^{-1}$ ) than all other treatments (Table 5.11).

Table 5.11. Effect of ethylene antagonists on individual and total organic acids of apple cv. 'Cripps Pink' after CA storage.

Ethylene antagonists	Organic acids (g kg <sup>-1</sup> )											
	Malic acid		Mean (T)	Succinic acid		Mean (T)	Citric acid		Mean (T)	Total organic acids		Mean (T)
	6M-CA	8M-CA		6M-CA	8M-CA		6M-CA	8M-CA		6M-CA	8M-CA	
Control	4.35±0.5a	5.19±0.0ab	4.77a	0.43±0.1bc	0.22±0.0ab	0.33b	3.57±0.5	4.95±0.6	4.26	8.6±0.9	10.4±0.6	9.5a
1-MCP	5.39±0.2ab	6.44±0.1bc	5.92ab	0.18±0.0ab	0.06±0.0a	0.12ab	6.87±0.9	8.43±0.8	7.65	12.7±1.0	15.2±0.9	13.9ab
1-HCP	7.93±0.2c	5.35±0.4ab	6.64b	0.08±0.0a	0.09±0.0a	0.09a	5.49±0.8	7.71±0.3	6.60	13.8±1.0	13.4±0.7	13.6ab
(S)-(-)-Lim	5.64±0.2ab	6.17±0.0abc	5.91ab	0.09±0.0a	0.54±0.0c	0.31b	9.39±1.1	7.98±1.1	8.68	15.4±1.1	14.9±1.0	15.2b
TCA	5.73±0.3ab	4.41±0.1a	5.07a	0.25±0.1abc	0.33±0.1abc	0.29b	5.97±0.2	7.29±0.6	6.63	12.2±1.0	12.3±0.6	12.3ab
Mean (SP)	5.81	5.51		0.21	0.25		6.26	7.27		12.5	13.3	
LSD ( $P \leq 0.05$ )	T = 1.3, SP = NS, T×SP = 1.8			T = 0.2, SP = NS, T×SP = 0.3			T = NS, SP = NS, T×SP = NS			T = 4.5, SP = NS, T×SP = NS		

6M-CA = storage in controlled atmosphere for 6 months, 8M-CA = storage in controlled atmosphere for 8 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM). T = treatment, SP = storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 5.12. Effect of ethylene antagonists on individual and total organic acids of apple cv. 'Fuji' after CA storage.

Ethylene antagonists	Organic acids (g kg <sup>-1</sup> ) Fuji (6M-CA)			
	Malic acid	Succinic acid	Fumaric acid	Total organic acids
Control	1.74±0.17	0.06±0.0	0.23±0.01	2.03±0.18
1-MCP	1.74±0.07	0.14±0.01	0.27±0.0	2.15±0.06
1-HCP	1.56±0.06	0.07±0.01	0.25±0.0	1.891±0.07
( <i>S</i> )-(-)-Lim	0.97±0.04	0.08±0.01	0.20±0.01	1.25±0.03
TCA	1.19±0.03	0.09±0.01	0.26±0.01	1.533±0.01
LSD ( $P \leq 0.05$ )	NS	NS	NS	NS

6M-CA = storage in controlled atmosphere for 6 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (*S*)-(-)-Lim = (*S*)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M), NS = Non-significant.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

**5.3.2.6. Ascorbic Acid, Total Phenolics and Antioxidant Capacity**

In ‘Cripps Pink’, mean ascorbic acid concentration was significantly higher with 1-MCP fumigation treatment (272.5 mg Kg<sup>-1</sup>) in comparison to all other treatments and control (Table 5.13). Following 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA treatments also resulted in significantly higher ascorbic acid level (223.7, 216.8 and 243.0 mg Kg<sup>-1</sup> respectively) than control. CA storage periods and their interaction with ethylene antagonists was non-significant for ascorbic acid concentration in ‘Cripps Pink’ apple fruit (Table 5.13).

The mean total phenolics were significantly higher in TCA, (*S*)-(-)-limonene and 1-MCP treated ‘Cripps Pink’ fruit (4200, 4017 and 4050 GAE mg Kg<sup>-1</sup> respectively) as compared to 1-HCP and control. Data for CA storage periods as well as for their interaction with ethylene antagonists was found to be non-significant for total phenolics in ‘Cripps Pink’ (Table 5.13).

Mean antioxidant capacity of ‘Cripps Pink’ fruit was significantly higher with 1-MCP and TCA treatments (68.5 and 68.8 mM Trolox Kg<sup>-1</sup> respectively) in comparison to all other treatments and control. Among CA storage periods, mean antioxidant concentration was significantly higher after 6 months CA storage (68.5 mM Trolox Kg<sup>-1</sup>) than 8 months (57.6 mM Trolox Kg<sup>-1</sup>). Interaction of ethylene antagonists and CA storage periods was significant for total antioxidant capacity of ‘Cripps Pink’. All fumigation treatments showed significantly higher antioxidant concentration than control after 6 months CA storage (66.9-72.6 mM Trolox Kg<sup>-1</sup>) while after 8 months CA storage, only 1-MCP and TCA treated ‘Cripps Pink’ fruit showed significantly higher antioxidant capacity (65.0 and 64.7 mM Trolox Kg<sup>-1</sup> respectively) than all other treatments and control (Table 5.13).

In ‘Fuji’ apple cultivar, 1-MCP was found to be superior as it led to significantly higher ascorbic acid concentration (243.7 mg Kg<sup>-1</sup>) in comparison to all other treatments and control after 6 months CA storage. 1-HCP and TCA treatments were parallel to 1-MCP and resulted in relatively higher ascorbic acid level (231.3 and 240.9 mg Kg<sup>-1</sup> respectively) than (*S*)-(-)-limonene and control. Effect of ethylene antagonists was non-significant for total phenolics in ‘Fuji’ after 6 months CA storage. TCA treated ‘Fuji’ fruit exhibited considerably higher antioxidant capacity (76.9 mM

Trolox  $\text{Kg}^{-1}$ ) than control and all other treatments after 6 months CA storage. Nonetheless, other fumigation treatments and control did not differ significant from TCA (Table 5.14).

Table 5.13. Effect of ethylene antagonists on ascorbic acid, total phenolics and total antioxidants of apple cv. 'Cripps Pink' after CA storage.

Ethylene antagonists	Ascorbic acid (mg Kg <sup>-1</sup> )		Mean (T)	Total phenolics (GAE mg kg <sup>-1</sup> )		Mean (T)	Total antioxidants (mM Trolox kg <sup>-1</sup> )		Mean (T)
	6M-CA	8M-CA		6M-CA	8M-CA		6M-CA	8M-CA	
Control	172.9±0.9	166.8±0.6	169.9a	3533±295.1	3200±178.2	3367ab	61.0±2.39cd	47.2±0.8a	54.1a
1-MCP	280.9±1.2	264.1±3.0	272.5d	3800±170.2	4300±108.1	4050bc	71.9±0.07e	65.0±0.09cde	68.5c
1-HCP	224.1±1.4	223.3±1.6	223.7b	3400±143.5	3100±209.7	3250a	66.9±0.26de	58.3±0.69bc	62.6b
(S)-(-)-Lim	218.3±4.8	215.3±4.7	216.8b	4033±36.0	4000±23.6	4017bc	69.9±0.41e	52.8±0.74ab	61.3b
TCA	245.6±1.2	240.4±1.2	243.0b	4233±13.6	4167±13.6	4200c	72.6±1.88e	64.7±0.86cde	68.8 c
Mean (SP)	228.4	221.9		3800	3753		68.5b	57.60a	
LSD ( $P \leq 0.05$ )	T = 12.3, SP = NS, T×SP = NS			T = 0.03, SP = NS, T×SP = NS			T = 5.4, SP = 3.4, T×SP = 7.6		

6M-CA = storage in controlled atmosphere for 6 months, 8M-CA = storage in controlled atmosphere for 8 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM). T = Treatment, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 5.14. Effect of ethylene antagonists on ascorbic acid, total phenolics and total antioxidants of apple cv. 'Fuji' after CA storage.

Ethylene antagonists	Fuji (6M-CA)		
	Ascorbic acid (mg Kg <sup>-1</sup> )	Total phenolics (GAE mg kg <sup>-1</sup> )	Total antioxidants (mM Trolox kg <sup>-1</sup> )
Control	181.4±1.75a	4067±36.0	60.5±2.19a
1-MCP	243.7±0.96c	4200±47.2	67.3±0.38ab
1-HCP	231.3±2.69bc	4267±27.2	62.9±0.26a
(S)-(-)-Lim	228.0±3.69b	4333±13.6	65.1±0.27ab
TCA	240.9±2.26bc	4067±36.0	76.9±2.11b
LSD ( $P \leq 0.05$ )	14.3	NS	11.57

6M-CA = storage in controlled atmosphere for 6 months, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M), NS = Non-significant.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$

#### **5.4. Discussion**

Fumigation treatments of ethylene antagonists greatly reduced the rate of ethylene production and delayed the onset of ethylene climacteric peak in ‘Cripps Pink’ and ‘Fuji’ apple after 6 and 8 months of CA storage. 1-MCP and 1-HCP treatments were found to be more effective in suppressing and delaying the ethylene climacteric peaks of both apple cultivars in comparison to all other treatments and control (Table 5.1 - 5.2).

Reduction and delay in climacteric ethylene peak with 1-MCP has also been reported earlier in plum cv. ‘Gulfruby’ and ‘Beauty’ (Abdi *et al.*, 1998), apple cv. ‘Golden Delicious’ (Janisiewicz *et al.*, 2003) and banana (Yan *et al.*, 2011). Estimation of ethylene production is an important parameter to predict loss of firmness in commercial storages. Highly reduced ethylene production by 1-MCP both in ‘Fuji’ and ‘Cripps Pink’ after long term CA storage is not surprising due to significantly reduced expression of ACO1 and ACS1 (ethylene biosynthesis genes) by 1-MCP in apples as previously documented by Yang *et al.* (2013). Moreover, Yan *et al.* (2011) reported that 1-MCP delays climacteric ripening in banana by suppressing the expression of MaACS1, MaERS2, MaERS3, MaEIL1, MaEIL3 and MaEIL4 genes which are associated with the ethylene signalling pathway. 1-HCP has also been found to suppress and delay the onset of climacteric ethylene peak earlier in banana (Sisler *et al.*, 2003) and tomato cv. ‘Kommeet’ (Khan *et al.*, 2016). 1-HCP is a substituted structural analogue of 1-MCP with 6 carbons and its efficacy could be due to an extended chain length as claimed by Sisler *et al.* (2003) that cyclopropenes with substitution at 1 carbon position have more competency as ethylene antagonists. These facts justify the reduced ethylene production and delayed climacteric peak in ‘Cripps Pink’ for 6 and 5.5 days effectively with 1-MCP and 1-HCP respectively as compared to (*S*)-(-)-limonene, TCA and control. Additionally, suppressed climacteric ethylene peak in ‘Cripps Pink’ after 8 months CA storage as compared to 6 months (Table 5.1) may be due to the post-climacteric stage after prolonged storage, which reduces the ability of fruit to biosynthesize ethylene by impairing the conversion of 1-aminocyclopropane 1-carboxylic acid (ACC) into ethylene (Argenta *et al.*, 2003). CA storage may have reduced the rate of ethylene production in both apple cultivars due to the inhibition of key enzyme activities involved in ethylene biosynthesis and the

conversion of ACC to ethylene by high carbon dioxide and low oxygen concentrations. Further, CO<sub>2</sub> has also been reported as an ethylene antagonist which helps to reduce autocatalytic ethylene biosynthesis (Beaudry, 1999; Wang and Sugar, 2013).

Respiration rate was suppressed by all ethylene antagonist treatments in ‘Fuji’ and ‘Cripps Pink’ after 6 and 8 months CA storage when estimated after 10 days of simulated shelf conditions. Specifically, 1-MCP reduced the respiration rate in ‘Cripps Pink’ significantly as compared to all other ethylene antagonists and control. However, the onset of respiratory climacteric peak in ‘Cripps Pink’ was also delayed by (*S*)-(-)-limonene and TCA following 1-MCP. While in ‘Fuji’, 1-HCP significantly delayed the respiratory climacteric peak similar to 1-MCP (Table 5.3 and 5.4). 1-MCP has been used extensively to delay ripening processes in climacteric fruits and reduced respiration rate in many horticultural crops including apple (Rupasinghe *et al.*, 2000; Bai *et al.*, 2005; Dal Cin *et al.*, 2006), plum (Martinez-Romero *et al.*, 2003) and papaya (Fabi *et al.*, 2007). Highly effective impact of 1-MCP in reducing rate of climacteric respiration rate is probably due to the blockage of autocatalytic ethylene production and ultimate reduction in the ethylene-dependent biosynthesis of new receptor sites (Blankenship and Dole, 2003; Dal Cin *et al.*, 2006). Reduced rate of respiration and delayed respiratory climacteric peak by 1-HCP, (*S*)-(-)-limonene and TCA also seem to be related with reduced ethylene production by these fumigation treatments. In addition, higher partial pressure of CO<sub>2</sub> in CA storage leads to a decline in rate of respiration and low temperature during CA storage also aids to reduce the concentration gradient of gases and ultimate respiration rate is lowered (Ho *et al.*, 2010).

Apple fruit colour is highly important from a commercial point of view as it affects consumer acceptability (Whale and Singh, 2007). Red colour of apple fruit including ‘Fuji’ and ‘Cripps Pink’ is due to anthocyanin pigments which also have health benefits due to their potential to scavenge free radicals (Awad and Jager, 2002). The red blush (a\*) was improved with 1-HCP and (*S*)-(-)-limonene, while lightness (L\*) and yellowness (b\*) was reduced with 1-MCP, 1-HCP and (*S*)-(-)-limonene in ‘Cripps Pink’ (Table 5.5 - 5.6). The peaks of anthocyanins are associated with the maturation phase of apple fruit when the concentration of ethylene begins to rise (Lister *et al.*, 1994; Awad *et al.*, 2001). However, Murphey and Dilley (1988) claimed

that only a slight exposure to ethylene is enough to start the biosynthesis of anthocyanins without influencing any other ripening parameters.

Loss of firmness is an important change associated with ripening as genes which are responsible for cell wall degradation respond primarily to ethylene (Bapat *et al.*, 2010). In the present study, all tested ethylene antagonists greatly inhibited the fruit softening in 'Fuji' and 'Cripps Pink', however, 1-MCP treated fruit exhibited higher firmness as compared to all other treatments and control (Table 5.7-5.8). Efficacy of ethylene antagonists in firmness retention may be ascribed to the reduced rates of ethylene production with fumigation treatments, which ultimately reduce the synthesis of pectin degrading proteins and thus inhibit the loss of fruit firmness (Gwanpua *et al.*, 2012). Moreover, the impact of ethylene inhibitors on quality of apple fruit depends upon storage periods as previously reported by Mattheis *et al.* (2005). The fruit firmness of 6-month CA stored 'Cripps Pink' apple was higher than 8 months CA stored fruit (Table 5.7). Bai *et al.* (2005) also found decline in fruit firmness of 'Gala', 'Delicious', 'Granny Smith' and 'Fuji' apple cultivars from 2 to 8 months of CA storage. Loss of firmness is generally slow during CA storage but increases sharply at shelf conditions due to variations in temperature and oxygen levels between storage and ambient conditions (Gwanpua *et al.*, 2012). TA and SSC are reliable indicators of apple fruit's acidity and sweetness. 1-MCP treated 'Cripps Pink' and 'Fuji' fruit showed relatively high TA and SSC as compared to all other treatments and control. Nonetheless, 1-HCP, (*S*)-(-)-limonene and TCA were also effective in maintaining fruit acidity and sweetness (Table 5.7-5.8). Bureau *et al.* (2012) reported that a difference of 0.08 - 1 % is required between TA and SSC of various fruit to judge the changes in their taste. According to this criteria, 1-HCP, (*S*)-(-)-limonene and TCA treated 'Fuji' and 'Cripps Pink' fruit in my results have no variation in terms of fruit taste as they have > 0.08 % difference in TA and SSC values. Sugar/acid ratio is maximum in control fruit of both 'Fuji' and 'Cripps Pink' after each storage period, which represents the perception of fruit sweetness by consumers (Contessa and Botta, 2016).

The concentration of ascorbic acid, total phenolics and total antioxidants is an indicator of apple's nutritional value (Wojdylo *et al.*, 2008). Moreover, these compounds are also involved in the defensive mechanism in plants and depend highly on genotypes/cultivars (Persic *et al.*, 2017). All fumigation treatments of ethylene

antagonists maintain relatively higher concentrations of ascorbic acid, total phenolics and total antioxidants in both apple cultivars as compared to the control (Table 5.13 - 5.14). The effect of CA storage periods was not significant for ascorbic acid and total phenolics in 'Cripps Pink'. However, generally low ascorbic acid concentration in 'Cripps Pink' after 8 months CA storage than 6 months stored fruit is consistent with the report of Lee and Kader (2000) that ascorbic acid normally declines in fruit with prolonged storage and exposure to excessive CO<sub>2</sub> which promotes oxidation of ascorbic acid.

Concentrations of individual and total sugars were influenced by ethylene antagonist treatments with comparatively higher concentration of glucose, fructose and total sugars in 'Cripps Pink' with 1-MCP treatment while in 'Fuji', these sugars were predominantly high with (S)-(-)-limonene treatment. After 6 months CA storage of 'Fuji', sucrose and glucose concentrations were almost parallel, but fructose concentration was comparatively higher than sucrose and glucose. In 'Cripps Pink', sucrose and glucose level increased after 8 months CA storage as compared to 6 month CA storage (Table 5.9 - 5.10). Similarly, Drake and Eisele (1999) also reported an increase in these sugars in 'Gala' apple after CA storage. However, fructose level reduced after 8 months CA storage in comparison to 6 months CA stored fruit of 'Cripps Pink' which can lead to a reduction in fruit sweetness as fructose is a sweeter sugar as compared to the sucrose and glucose (Contessa and Botta, 2016). How these ethylene antagonists affect the sugar metabolism during CA storage of apple fruit is not exactly known and warrants investigation.

Among individual organic acids which were identified and quantified by HPLC, malic acid was higher in concentration in both apple cultivars regardless of CA storage period as malic acid comprise 90 % of apple's acid content while others are present in trace amounts (Ackermann *et al.*, 1992). Citric acid was almost parallel in concentration to malic acid in 'Cripps Pink' after 6 and 8 months CA storage; however, it was not found in 'Fuji' fruit after 6 months CA storage. In general, the changes in organic acid concentrations were non-significant with ethylene antagonist and storage periods probably because of reduced respiration rate by fumigation treatments; since, respiratory metabolism utilize organic acids as a substrate (Ackermann *et al.*, 1992).

The variation in responses to tested ethylene antagonists in this study may be attributed to the differential effect of these compounds on expression of ripening related genes and turnover of ethylene receptors after fumigation (Ziliotto *et al.*, 2008). However, all the treatments maintained the fruit quality in both apple cultivars to varying levels. Efficacy of (*S*)-(-)-limonene and TCA in extending CA storage life and quality of ‘Fuji’ and ‘Cripps Pink’ advocate that compounds from a plant’s essential oils are intensely lipophilic and have low molecular weight, which increase their permeability through cell membranes for biological activity (Solgi and Ghorbanpour, 2014). In conclusion, 1-HCP, (*S*)-(-)-limonene and TCA can be used as effective ethylene antagonists to minimise the drawbacks of 1-MCP application in CA storage of apple fruit.

## CHAPTER 6

### Effect of Ethylene Antagonists on Cold Storage Life and Fruit Quality of Pear cv. 'Packham's Triumph' and 'Beurre Bosc'

#### Abstract

Pear is a typical climacteric fruit and commercial growers face critical postharvest challenges during its handling, storage and transportation to keep fruit in acceptable quality for markets. These challenges are posed by a burst in ethylene production during climacteric fruit ripening. The purpose of the present research study was to evaluate the potential of putative ethylene antagonists to extend cold storage life and maintain postharvest quality of pear fruit. The pear fruit cv. 'Packham's Triumph' and 'Beurre Bosc' were fumigated with 1-methylcyclopropene (1-MCP, 740 ppb), 1-hexylcyclopropene (1-HCP, 1  $\mu\text{M}$ ), (*S*)-(-)-limonene (1  $\mu\text{M}$ ) and *trans*-cinnamaldehyde (TCA, 1  $\mu\text{M}$ ) to probe their efficacy on the extension of cold storage life and quality of these pear cultivars in two independent experiments. Fruit without any fumigation treatment were kept as control. Following the treatments with ethylene antagonists, the pear fruit were stored at 0 - 1 °C and  $> 85 \pm 5$  % R.H. for the period of 4 and 6 months. Following each cold storage period, the fruit were transferred to simulated shelf conditions for 10 days and assessed for ethylene production and respiration rate until the climacteric phase was approached. Moreover, the fruit quality and physico-chemical parameters were determined at 10<sup>th</sup> day of simulated shelf conditions. Various parameters of pear fruit quality were affected by ethylene antagonists and cold storage periods, however, the effect of 1-MCP was generally better as compared to other ethylene antagonists and the control. Climacteric ethylene production peak was highly suppressed in 'Packham's Triumph' and 'Beurre Bosc' with 1-MCP (20.9 and 71.6  $\text{pmol kg}^{-1} \text{s}^{-1}$  respectively) followed by (*S*)-(-)-limonene in 'Beurre Bosc' (307.7  $\text{pmol kg}^{-1} \text{s}^{-1}$ ). All fumigation treatments delayed the onset of climacteric ethylene peaks (1.83 - 3 days) in both pear cultivars as compared to the control. The rate of climacteric respiration peak was highly reduced by 1-MCP in 'Packham's Triumph' and 'Beurre Bosc' (0.33 and 0.30  $\mu\text{mol kg}^{-1} \text{s}^{-1}$  respectively) as compared to all other fumigants and control. Meanwhile, onset of respiratory peak was delayed by 1-MCP and 1-HCP treatments (2.83 and 2.33 days respectively) in 'Beurre Bosc' pear. Similarly, fruit firmness, total antioxidant capacity and ascorbic acid concentration were significantly higher in 1-MCP fumigated fruit in both pear

cultivars. The fumigation of 1-HCP, (*S*)-(-)-limonene and TCA also maintained the firmness, ascorbic acid concentration and total antioxidants as compared to the control in both pear cultivars. Titratable acidity (TA) was higher with 1-MCP fumigation in ‘Packham’s Triumph’ (0.36 %) and ‘Beurre Bosc’ (0.37 %) pears while soluble solids concentration (SSC) and SSC/TA ratio was significantly higher in control ‘Beurre Bosc’ fruit (14.9 % and 116.0 respectively). The concentrations of individual and total organic acids were not substantially affected by fumigation treatments and cold storage periods. The individual and total sugars of both pear cultivars were significantly affected by the ethylene antagonists and cold storage durations but did not show any specific trend. These experimental findings suggest that although 1-MCP exhibited the most effective results, 1-HCP, (*S*)-(-)-limonene and TCA have potential as well to act as ethylene antagonist for suppressing the production of climacteric ethylene, respiration rate and to maintain the fruit quality of pears during medium and long term cold storage.

### 6.1. Introduction

Pear (*Pyrus communis* L.) is regarded as a valuable fruit for its delicious flavour and health benefits including improved digestion, immune system, reduced blood pressure and prevention from heart diseases (Salta *et al.*, 2010; Lee *et al.*, 2011). Pear fruit has been found to be a rich source of flavonoids, amino acids, antioxidants and phenolic compounds which are responsible for its beneficial impact on human health (Kou *et al.*, 2014). On the global scale, China is the leading producer of pears (FAOSTAT, 2016). In Australia, total production of pears (including nashi) is 104928 tonnes (ABS, 2017) with 89 % contribution from the state of Victoria (APAL, 2018).

Australian pear cultivars are diverse in shape, colour, size and taste which are available in markets between early autumn season and summer (AgriFutures Australia, 2017; APAL, 2018). Cold storage is one of the techniques practiced commercially to extend the postharvest life of pear fruit. Pear is a climacteric fruit and its ripening is associated with a sharp increase in autocatalytic production of ethylene and respiration rate (Larrigaudiere *et al.*, 2004). Hence, pears have a short ripening period due to accelerated physiological changes after harvesting which critically affects its storage life, quality attributes and poses considerable market challenges (Kou *et al.*, 2014). Moreover, cold temperatures (-1 to 1 °C) during storage stimulate an excessive fruit

softening in pear after shifting fruit to the shelf conditions. Hence, to increase consumer acceptance and to fetch higher prices, quality of pears needs to be maintained during and after cold storage (Lopez *et al.*, 2011).

Ethylene is a ripening hormone which plays a key role in promoting ripening process including fruit colour, flavour, softening and other physico-chemical attributes (Lelievre *et al.*, 1997; Hiwasa *et al.*, 2003). Presence of ethylene in the supply chain further hastens ripening of climacteric fruits and leads to fruit decay and postharvest losses (Blanke, 2014; Sánchez *et al.*, 2017). Similarly, the exposure of pear fruit to ethylene during cold storage leads to rapid ripening, fruit softening and various ethylene induced disorders like internal browning, skin yellowing and scalds (Wills and Warton, 2000; Bower *et al.*, 2003; Gapper *et al.*, 2006). Various reports have been documented in literature on the prevention of fruit from adverse effects of ethylene in pear (Trincherro *et al.*, 2004; Li *et al.*, 2013; Argenta *et al.*, 2016) and other climacteric fruits (Ortiz *et al.*, 2005; Ergun *et al.*, 2005; do Nascimento *et al.*, 2006; Menniti *et al.*, 2006; Huber, 2008; Xu *et al.*, 2014 and 2016). It has been reported that there is a possibility to overcome the above-mentioned postharvest issues by preventing ethylene exposure and through precise control of storage atmosphere (Singh *et al.*, 2009). However, in commercial storages, where fruits are closely packed in bulk, keeping ethylene concentration at a highly reduced level is a challenging task. Hence, this situation demands new approaches to inhibit ethylene perception by fruit during storage to prevent quality losses and storage disorders (Bower *et al.*, 2003).

Postharvest technology offers innovative approaches to reduce losses in fresh horticultural produce to prevent malnutrition and to feed the ever-increasing world population (Mahajan *et al.*, 2014). For instance, inhibiting ethylene biosynthesis by using aminoethoxyvinylglycine (AVG) which retards the conversion of S-adenosyl methionine to 1-aminocyclopropane-1-carboxylic acid (ACC), a critical step in ethylene biosynthesis. AVG has been found to delay ripening in various cultivars of pear (Romani *et al.*, 1982 and 1983; Clayton *et al.*, 2000). Similarly, carbon dioxide (CO<sub>2</sub>) has also been reported as an inhibitor of ethylene biosynthesis in pear fruit (Chavez-Franco and Kader, 1993; de Wild *et al.*, 2003). However, inhibition of ethylene biosynthesis is not an ideal approach as it does not protect the fruits from exogenous sources of ethylene (Lau and Yang, 1976; Baker *et al.*, 1978; Reid and Wu, 1992; Martinez-Romero *et al.*, 2007). Inhibition of ethylene action by using ethylene

antagonists to prevent deleterious effects of ethylene in fruit is more effective than inhibition of ethylene biosynthesis (Ansari and Tuteja, 2015). Ethylene antagonists are an effective tool to retard the ripening process and minimise fruit quality losses by providing protection from both endogenous and exogenous sources of ethylene (Pirrung *et al.*, 2008). These compounds bind to the ethylene receptors either reversibly or irreversibly, preventing the downstream signal transduction pathways (Goren *et al.*, 2008).

1-MCP is an ethylene antagonist, commonly used commercially to keep horticultural commodities fresh for longer periods by inhibiting ethylene action (Zhu *et al.*, 2015). Previously, 1-MCP has been reported to delay fruit ripening, softening and senescence in pear (Kubo *et al.*, 2003; Hiwasa *et al.*, 2003; Ekman *et al.*, 2004; Mwaniki *et al.*, 2005; Spotts *et al.*, 2007; Calvo and Sozzi, 2009; Villalobos-Acuna *et al.*, 2011). Postharvest benefits of 1-MCP application in pear are valuable; however, the efficacy of 1-MCP has been reported to depend upon genotype, ripening parameters and maturity stage (Huber, 2008). Some previous research studies report irregular colour changes and variation in maturity stages of 1-MCP treated fruit (Mir *et al.*, 2001; Cameron and Reid, 2001; Xu *et al.*, 2014). Moreover, some pear cultivars fail to attain normal ripening and softening following treatment with 1-MCP (Villalobos-Acuna *et al.*, 2011). These shortcomings of 1-MCP necessitate the screening of some new compounds as ethylene antagonists for the horticulture industry. Structural analogues of 1-MCP have been synthesized by Sisler *et al.* (2003) which inhibit the action of ethylene. It has been reported by various researchers that substituted cyclopropenes are effective to inhibit the adverse effects of ethylene (Feng *et al.*, 2000 and 2004; Apelbaum *et al.*, 2008; Xu *et al.*, 2014 and 2016). 1-HCP is one of these structural analogues which has substituted side chain at 1-carbon position and can elicit ethylene inhibitory effects similar to 1-MCP (Serek *et al.*, 2007; Khan *et al.*, 2016; Abdalghani, 2017). There are some other novel compounds which can also be used as ethylene antagonists. Sisler *et al.* (2006) have mentioned limonene and TCA as ethylene antagonists from plant origins. Limonene belongs to the group of natural products called 'Terpenes' which have potential to compete for ethylene receptor sites (Grichko *et al.*, 2003). Similarly, TCA is a compound from cinnamon oil which has been reported to maintain the quality attributes of fresh produce and reduce the microbial degeneration of fruits/vegetables due to its antimicrobial activity (Jing *et al.*,

2011; Carvalho *et al.*, 2016). Recently, natural products are gaining popularity due to increasing concerns of the public regarding the perceived toxic effects of synthetic chemicals on environment and human health (Ibrahim *et al.*, 2004).

Hence, the present research study was designed to evaluate the efficacy of 1-HCP, (*S*)-(-)-limonene and TCA in comparison to 1-MCP to extend the cold storage life and maintain quality of pear cultivars i.e. ‘Packham’s Triumph’ and ‘Beurre Bosc’.

### 6.2. Materials and Methods

#### 6.2.1. Experiments

There were two independent experiments in this research study to evaluate the potential of ethylene antagonists for the extension of storability in cold storage conditions and keeping of fruit quality of pear cv. ‘Packham’s Triumph’ and ‘Beurre Bosc’. Experiment details are as follows.

##### 6.2.1.1. Experiment 1: Effect of ethylene antagonists on cold storage life and fruit quality of pear cv. ‘Packham’s Triumph’.

In this experiment, ‘Packham’s Triumph’ pear fruit were fumigated with four different ethylene antagonists i.e., 1-MCP (740 ppb), 1-HCP (1  $\mu$ M), (*S*)-(-)-limonene (1  $\mu$ M) and TCA (1  $\mu$ M). Fruit which did not receive any treatment were kept as a control. The experiment was designed by following two factor factorials (ethylene antagonists  $\times$  cold storage periods) completely randomized design (CRD). Each treatment was replicated 3 times with 20 fruit in each replication.

After fumigation with ethylene antagonists, both treated and untreated (control) ‘Packham’s Triumph’ fruit were stored at 0 - 1 °C and  $> 85 \pm 5$  % R.H. for the period of 4 and 6 months. After cold storage periods, the pear fruit were moved to simulated shelf conditions ( $21 \pm 1$  °C) and ethylene production and respiration rate were recorded daily until climacteric peaks were obtained. Various quality parameters *viz.* firmness, TA, SSC, SSC/TA ratio, individual and total sugars, organic acids, ascorbic acid and total antioxidants were analysed on 10<sup>th</sup> day of simulated shelf conditions. Temperature and R.H. were monitored during cold storage using Tinytags (Gemini Data Loggers, West Sussex, UK) operated by Tinytag Explorer Software.

**6.2.1.2. Experiment 2: Effect of ethylene antagonists on cold storage life and fruit quality of pear cv. 'Beurre Bosc'.**

This experiment was conducted to evaluate the efficacy of 1-MCP (740 ppb), 1-HCP (1  $\mu$ M), (*S*)-(-)-limonene (1  $\mu$ M) and TCA (1  $\mu$ M) fumigation treatments on cold storage life and fruit quality of 'Beurre Bosc' pear. Untreated fruit served as a control. Experimental design was two factors CRD (ethylene antagonists  $\times$  cold storage periods) with three replications and 20 fruit in each replication. After fumigation, the 'Beurre Bosc' fruit were stored in cold rooms (0 - 1 °C and  $> 85 \pm 5$  % R.H.) for the period of 4 and 6 months. Ethylene production, respiration rate and fruit quality parameters were determined as mentioned above in experiment 1.

**6.2.2. Fruit Source**

Economically important cultivars of pear *viz.*, 'Packham's Triumph' and 'Beurre Bosc' were obtained from Newton Brother's orchards located in Manjimup, WA. Geographically, Manjimup is located at the latitude of 34°14' South and longitude of 116° 8' East. The fruit of both pear cultivars at commercial maturity with uniform size, free from visual symptoms of diseases and physical injuries were used in the experiments.

The pear fruit of both cultivars were transported immediately after harvesting to the Horticulture Research Laboratory, School of Molecular and Life Sciences, Curtin University, Bentley Campus, Perth, WA, where experiments were conducted including the application of fumigation treatments, cold storage and estimation of ethylene production, respiration rate and other fruit quality parameters.

**6.2.3. Ethylene Antagonists and their Sources**

Four different ethylene antagonists were used in these experiments namely 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA. Commercial formulation of 1-MCP (SmartFresh™) was obtained from AgroFresh, Victoria, Australia, while 1-HCP was synthesised by the research team of Dr. Alan Payne, School of Molecular and Life Sciences, Curtin University. The other ethylene antagonists i.e. (*S*)-(-)-limonene and TCA were purchased from ACROS Organic, Thermo Fisher Scientific, Scoresby, Victoria, Australia.

#### 6.2.4. Fumigation Treatment of Pear Fruit

Pear fruit were treated with 1-MCP, 1-HCP, (S)-(-)-limonene and TCA. For this purpose, fruit were fumigated with 1  $\mu$ M solution of ethylene antagonists in sealed plastic containers of 60 L volume for 24 hours. Soda lime (25 g) and a small fan was also kept inside containers to absorb CO<sub>2</sub> gas and to disseminate chemical fumes of ethylene antagonists respectively. The fruit were kept in sealed drums for 24 hours at room temperature. For 1-MCP fumigation treatment, tablets of commercial formulation (SmartFresh™ from AgroFresh, Victoria, Australia) were used having final concentration of 740 ppb and were dipped in buffer solution inside the containers. After fumigating the pear fruit cv. 'Packham's Triumph' and 'Beurre Bosc' with ethylene antagonists, both treated and untreated (control) fruit were stored in cold rooms at 0 - 1 °C and > 85 ± 5 % R.H. for the period of 4 and 6 months. After each cold storage period, the pear fruit were moved to simulated shelf conditions (21 ± 1 °C) and were analysed for ethylene production, respiration rate and fruit quality parameters.

#### 6.2.5. Determination of Ethylene Production, Respiration Rate and Fruit Quality

##### 6.2.5.1. Rate of ethylene production

To estimate the rate of ethylene production of pear fruit, a laser-based system of Sensor Sense (ETD-300, Nijmegen, Netherlands) was used. This procedure involved incubation of two uniform sized fruit from each replication in 1L volume jars. Jars were closed by rubber septum (SubaSeal®, Sigma-Aldrich Co., St. Louis, USA) and connected to ethylene detector system. Flow rate was set to 4 L after checking output channels for any blockage. Running time of samples was 20 min and concentration of ethylene was expressed as pmol kg<sup>-1</sup> s<sup>-1</sup> (Cristescu *et al.*, 2013).

##### 6.2.5.2. Rate of respiration

Respiration rate of pear fruit was determined as production of CO<sub>2</sub> gas using an infrared gas analyser (Servomex Series 1400, Sussex, UK). For this purpose, two pear fruit of uniform size and weight were incubated in sealed glass jars (1 L) sealed with rubber septum for 1-2 hours. After incubation period, concentration of CO<sub>2</sub> gas was determined from the head space gas using an infrared gas analyser. The rate of respiration was expressed as  $\mu$ mol kg<sup>-1</sup> s<sup>-1</sup>.

#### **6.2.5.3. Fruit firmness**

Texture analyser (TA Plus, Lloyds Instruments, Hampshire, UK) connected with 11.1 mm diameter plunger, operated by Nexygen™ Plus Material Testing Software was used to assess the firmness of pear fruit. Firmness was determined on two opposite sides of the fruit and was expressed as Newtons (N). A diagram for detailed explanation of firmness determination by texture analyser has been given in Chapter 3, Section 3.7.4.

#### **6.2.5.4. TA, SSC and SSC/TA ratio**

TA of pear fruit was estimated by titration method. Mixture of fruit juice (10 ml) from each replication was diluted with distilled water (20 ml) and an aliquot (5 ml) was titrated against 0.1N NaOH solution. Phenolphthalein was used as an indicator to show the end point of titration by the development of pink colour. TA was calculated by a formula as described in Chapter 3, Section 3.7.3 and was expressed as a malic acid percentage (%).

Refractometer (ATAGO™ Digital Palette Refractometer, PR-101, Tokyo, Japan) was used to determine SSC from juice samples of each replicate and expressed as a %. For the calculation of SSC/TA ratio, the values of SSC (%) were divided by corresponding TA (%) values.

#### **6.2.5.5. Total and individual sugars and organic acids**

The reverse phase liquid chromatography using HPLC system (Waters, 717plus, Milford Corp, MA, USA) was used to determine the concentrations of individual sugars and organic acids in pear pulp according to the method of Khan *et al.* (2016). Details of HPLC system and analytical procedure are given in Chapter 3, Section 3.7.9.

#### **6.2.5.6. Ascorbic acid**

Ascorbic acid concentration in pear fruit was determined according to the previously reported method of Wan Zaliha (2009) with some alterations as detailed in Chapter 3, Section 3.7.6.

#### **6.2.5.7. Total antioxidants**

DPPH assay was used to determine the concentration of total antioxidants in pear fruit according to the protocol of Brand-Williams *et al.* (1995) with some modifications as described in Chapter 3, Section 3.7.8.

### **6.2.6. Statistical Analysis**

Data were statistically analysed using software package of Genstat, version 14.1 (VSN International Ltd, Hemel Hempstead, UK). Two-way analysis of variance (ANOVA) was used to assess the effect of ethylene antagonists and cold storage periods on various fruit quality parameters. Moreover, least significant difference test (LSD) was applied to rank treatment means at  $P \leq 0.05$ .

## **6.3. Results**

### **6.3.1. Rate of Ethylene Production**

The rate of climacteric ethylene production and the onset of ethylene climacteric peaks were significantly affected by ethylene antagonists and cold storage periods in ‘Packham’s Triumph’ and ‘Beurré Bosc’ pear cultivars (Table 6.1 and 6.2). Mean rate of climacteric ethylene peak in ‘Packham’s Triumph’ pear was significantly suppressed by 1-MCP fumigation treatment ( $20.9 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ) followed by 1-HCP ( $595.5 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ) as compared to all other fumigation treatments and control (Table 6.1). The other ethylene antagonists, (*S*)-(-)-limonene and TCA also effectively reduced the climacteric ethylene peak ( $727.9$  and  $664.9 \text{ pmol kg}^{-1} \text{ s}^{-1}$  respectively) in comparison to control ( $888.0 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ). Mean rate of ethylene production at climacteric peak was significantly less after 6 months of CS ( $352.3 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ) than 4 months CS ( $806.6 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ). The interaction between ethylene antagonist fumigation treatments and cold storage periods was also found to be significant for the rate of climacteric ethylene production in ‘Packham’s Triumph’. 1-MCP treated fruit of ‘Packham’s Triumph’ resulted in significantly reduced rate of climacteric ethylene production after 4 and 6 months CS ( $14.9$  and  $26.9 \text{ pmol kg}^{-1} \text{ s}^{-1}$  respectively). In addition, 1-HCP fumigated fruit also had significantly suppressed climacteric ethylene peak after 6 months CS ( $236.0 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ) in comparison to all other fumigation treatments and control (Table 6.1).

In ‘Packham’s Triumph’ pear, 1-MCP fumigation significantly delayed the mean number of days to the onset of ethylene climacteric peak (2.83 days), followed

by a delay of 2 and 1.83 days with (*S*)-(-)-limonene and TCA fumigation treatments respectively in comparison to control (Table 6.1). The cold storage periods did not significantly affect the onset of ethylene climacteric peak in ‘Packham’s Triumph’. However, the interaction between ethylene antagonist treatments and cold storage periods was found to be significant for this parameter. All the fumigation treatments of ethylene antagonists considerably delayed the ethylene climacteric peak by 0.67 - 1.67 days as compared to the control in 4 months cold stored fruit. After 6 months CS of ‘Packham’s Triumph’ pear, the 1-MCP fumigated fruit exhibited a significant delay in climacteric ethylene peak by 4.33 days in comparison to control. Following 1-MCP, (*S*)-(-)-limonene and TCA fumigation treatments also delayed ethylene climacteric peaks by 2.33-3 days in 6 months cold stored fruit when compared with control (Table 6.1).

In ‘Beurré Bosc’ pear, mean rate of climacteric ethylene production was significantly reduced by 1-MCP fumigation ( $71.6 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ) followed by (*S*)-(-)-limonene ( $307.7 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ) as compared to all other fumigation treatments and control (Table 6.2). Mean rate of climacteric ethylene production in ‘Beurré Bosc’ was significantly reduced in 6 months cold stored fruit ( $307.13 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ) than 4 months ( $493.68 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ). The interaction between ethylene antagonists and cold storage periods was found to be significant for the rate of climacteric ethylene production. Relatively reduced rate of ethylene production at climacteric peak was noted in 1-MCP fumigated ‘Beurré Bosc’ fruit after 4 and 6 months CS ( $44.5$  and  $98.7 \text{ pmol kg}^{-1} \text{ s}^{-1}$  respectively) followed by (*S*)-(-)-limonene fumigation treatment ( $198.6 \text{ pmol kg}^{-1} \text{ s}^{-1}$ ) after 4 months CS (Table 6.2).

A significant delay in mean number of days to the onset of ethylene climacteric peak was recorded with 1-MCP, (*S*)-(-)-limonene and TCA fumigation treatments (3, 2.16 and 2.33 days respectively) as compared to the control in ‘Beurré Bosc’. 1-HCP fumigation treatment was statistically similar to control (Table 6.2). Cold storage periods did not significantly affect the mean number of days to the onset of ethylene peak. Ethylene antagonist treatments exhibited a significant interaction with cold storage periods for this parameter. Comparatively, delayed ethylene climacteric peaks were recorded in (*S*)-(-)-limonene fumigated ‘Beurré Bosc’ fruit after 4 months CS (4.67 days) while, in 1-MCP and TCA fumigated fruit (3 and 1.34 days respectively) after 6 months CS (Table 6.2).

Table 6.1. Effects of ethylene antagonist's fumigation on peak rate of ethylene production and number of days to the onset of ethylene climacteric peak in pear cv. 'Packham's Triumph' after 4 and 6 months of cold storage.

Ethylene antagonists	Ethylene Climacteric					
	Peak Rate (Ethylene pmol kg <sup>-1</sup> s <sup>-1</sup> )		Mean (T)	Onset (days)		Mean (T)
	4 M	6 M		4 M	6 M	
Control	1100.3±6.9f	675.8±7.3d	888.0e	5.33±0.3bc	4.00±0.0a	4.67a
1-MCP	14.9±0.8a	26.9±1.3a	20.9a	6.67±0.1d	8.33±0.1e	7.50c
1-HCP	955.0±9.8e	236.0±16.1b	595.5b	6.00±0.0cd	4.67±0.4ab	5.33a
(S)-(-)-Lim	1072.2±17.2f	383.6±4.7c	727.9d	7.00±0.0d	6.33±0.1cd	6.67b
TCA	890.5±6.1e	439.4±5.2c	664.9c	6.00±0.0cd	7.00±0.0d	6.50b
Mean (SP)	806.6b	352.3a		6.20	6.07	
LSD ( $P \leq 0.05$ )	T = 46.73	SP = 29.56	T × SP = 66.09	T = 0.83	SP = NS	T × SP = 1.18

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 μM), (S)-(-)-Lim = (S)-(-)-limonene (1 μM), TCA = *trans*-cinnamaldehyde (1 μM), T = Treatments, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 6.2. Effects of ethylene antagonist's fumigation on peak rate of ethylene production and number of days to the onset of ethylene climacteric peak in pear cv. 'Beurre Bosc' after 4 and 6 months of cold storage.

Ethylene antagonists	Ethylene Climacteric					
	Peak Rate (Ethylene pmol kg <sup>-1</sup> s <sup>-1</sup> )		Mean (T)	Onset (days)		Mean (T)
	4 M	6 M		4 M	6 M	
Control	981.2±4.5g	404.2±2.3e	692.7d	3.00±0.0a	5.33±0.1bc	4.167a
1-MCP	44.5±4.1a	98.7±2.8ab	71.6a	6.00±0.4bcd	8.33±0.1e	7.167b
1-HCP	664.7±9.6f	261.4±6.7cd	463.0c	5.00±0.0bc	4.667±0.3ab	4.833a
(S)-(-)-Lim	198.6±4.1bc	416.9±36.5e	307.7b	7.67±0.3de	5.00±0.0bc	6.333b
TCA	579.4±26.1f	354.4±2.4de	466.9c	6.33±0.3bcd	6.67±0.4cde	6.500b
Mean (SP)	493.68b	307.13a		5.60	6.00	
LSD ( $P \leq 0.05$ )	T = 81.7	SP = 51.6	T × SP = 115.5	T = 1.23	SP = NS	T × SP = 1.74

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = Treatments, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

**6.3.2. Rate of Respiration**

In 'Packham's Triumph' pear, 1-MCP fumigation suppressed the mean rate of respiratory peak significantly ( $0.332 \mu\text{mol kg}^{-1} \text{s}^{-1}$ ) in comparison to all other fumigation treatments and control (Table 6.3). 1-HCP, (*S*)-(-)-limonene and TCA fumigation also reduced the respiration rate at climacteric peak ( $0.497 - 0.561 \mu\text{mol kg}^{-1} \text{s}^{-1}$ ) in comparison to control ( $0.601 \mu\text{mol kg}^{-1} \text{s}^{-1}$ ). Mean rate of respiration at climacteric peak was significantly reduced after 6 months CS ( $0.407 \mu\text{mol kg}^{-1} \text{s}^{-1}$ ) than 4 months CS ( $0.608 \mu\text{mol kg}^{-1} \text{s}^{-1}$ ). The interaction between ethylene antagonists and cold storage periods was found to be non-significant for the peak rate of respiration in 'Packham's Triumph'. Similarly, the effect of ethylene antagonists, cold storage periods and their interaction was found to be non-significant for the number of days to the onset of respiratory climacteric peak in 'Packham's Triumph' pear (Table 6.3).

In 'Beurré Bosc' pear, all the fumigation treatments resulted in comparatively reduced mean rate of respiration at climacteric peak ( $0.301 - 0.588 \mu\text{mol kg}^{-1} \text{s}^{-1}$ ) as compared to the control ( $0.730 \mu\text{mol kg}^{-1} \text{s}^{-1}$ ). However, the effect of 1-MCP was more pronounced in reducing respiration rate than all other ethylene antagonists tested (Table 6.4). The effect of cold storage periods as well as their interaction with ethylene antagonists was noted to be non-significant for the rate of respiratory climacteric peak. Mean number of days to the onset of respiratory climacteric in 'Beurré Bosc' were delayed with 1-MCP, 1-HCP and TCA fumigation treatments by 2.83, 2.33 and 1.33 days respectively in comparison to control (Table 6.4). Mean days to the onset of respiratory climacteric were significantly less after 4 months (4.87 days) than 6 month CS (5.93 days) of 'Beurré Bosc'.

Table 6.3. Effects of ethylene antagonist's fumigation on climacteric peak rate of respiration (CO<sub>2</sub>) and number of days to the onset of respiratory climacteric peak in pear cv. 'Packham's Triumph' after 4 and 6 months of cold storage.

Ethylene antagonists	Respiratory Climacteric					
	Peak rates (CO <sub>2</sub> μmol kg <sup>-1</sup> s <sup>-1</sup> )		Mean (T)	Onset (days)		Mean (T)
	4 M	6 M		4 M	6 M	
Control	0.714±0.01	0.487±0.02	0.601c	6.00±0.0	4.33±0.27	5.167
1-MCP	0.416±0.00	0.247±0.01	0.332a	7.00±0.41	6.00±0.47	6.500
1-HCP	0.693±0.03	0.400±0.02	0.547bc	5.00±0.24	6.67±0.14	5.833
(S)-(-)-Lim	0.640±0.01	0.481±0.01	0.561bc	6.33±0.27	4.67±0.49	5.500
TCA	0.576±0.02	0.418±0.01	0.497b	4.67±0.27	6.00±0.41	5.333
Mean (SP)	0.608b	0.407a		5.80	5.53	
LSD ( $P \leq 0.05$ )	T = 0.085 SP = 0.054 T × SP = NS			T = NS	SP = NS	T × SP = NS

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 μM), (S)-(-)-Lim = (S)-(-)-limonene (1 μM), TCA = *trans*-cinnamaldehyde (1 μM), T = Treatments, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 6.4. Effects of ethylene antagonist's fumigation on climacteric peak rate of respiration (CO<sub>2</sub>) and number of days to the onset of respiratory climacteric peak in pear cv. 'Beurre Bosc' after 4 and 6 months of cold storage.

Ethylene antagonists	Respiratory Climacteric					
	Peak rates (CO <sub>2</sub> μmol kg <sup>-1</sup> s <sup>-1</sup> )		Mean (T)	Onset (days)		Mean (T)
	4 M	6 M		4 M	6 M	
Control	0.82±0.06	0.64±0.01	0.730c	3.00±0.00	5.00±0.24	4.00a
1-MCP	0.28±0.01	0.33±0.01	0.301a	7.00±0.00	6.67±0.36	6.83d
1-HCP	0.45±0.01	0.46±0.00	0.443ab	6.00±0.00	6.67±0.14	6.33cd
(S)-(-)-Lim	0.47±0.02	0.53±0.03	0.502b	3.33±0.14	5.67±0.14	4.50ab
TCA	0.65±0.05	0.52±0.02	0.588bc	5.00±0.00	5.67±0.49	5.33bc
Mean (SP)	0.532	0.493		4.87b	5.93a	
LSD ( $P \leq 0.05$ )	T = 0.15	SP = NS	T × SP = NS	T = 1.16	SP = 0.74	T × SP = NS

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 μM), (S)-(-)-Lim = (S)-(-)-limonene (1 μM), TCA = *trans*-cinnamaldehyde (1 μM), T = Treatments, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

**6.3.3. Fruit Firmness**

Fruit firmness was significantly influenced by the fumigation treatments of ethylene antagonists and cold storage periods in pear cv. 'Packham's Triumph' and 'Beurré Bosc' (Table 6.5). Mean fruit firmness of 'Packham's Triumph' pear was significantly higher with 1-MCP fumigation treatment (64.7 N) followed by TCA (34.1 N) in comparison to all other ethylene antagonist treatments and control. Significantly higher mean fruit firmness was observed after 4 months (38.3 N) than 6 months cold stored fruit (34.2 N). The interaction between ethylene antagonists and cold storage periods (4 and 6 months) was found to be significant for fruit firmness of 'Packham's Triumph'. After 4 months CS, significantly firmer fruit were observed in 1-MCP fumigation treatment (69.3 N) followed by TCA (36.0 N) and (*S*)-(-)-limonene (32.9 N). 1-MCP fumigated 'Packham's Triumph' fruit also exhibited significantly higher fruit firmness (59.9 N) after 6 months CS. In general, all ethylene antagonist treatments were effective to retain the firmness of 'Packham's Triumph' fruit after 4 and 6 months CS in comparison to control (Table 6.5).

In 'Beurré Bosc' pear, the mean fruit firmness was significantly higher when fumigated with 1-MCP (60.6 N) followed by 1-HCP (50.1 N). Significantly greater mean fruit firmness was recorded after 4 months CS of 'Beurré Bosc' pear (46.5 N) than 6 months (38.8 N). The interaction between ethylene antagonists and cold storage periods was significant for fruit firmness. All the fumigation treatments inhibited the loss of firmness to varying extents, however, 1-MCP fumigated 'Beurré Bosc' fruit were significantly firmer (73.2 N) followed by 1-HCP fumigation (52.7 N) after 4 months CS in comparison to all other fumigation treatments and control. Similarly, after 6 months CS, slightly higher fruit firmness was recorded in 1-MCP and 1-HCP fumigated 'Beurré Bosc' fruit (48.1 and 47.4 N) as compared to other fumigation treatments and control (Table 6.5).

Table 6.5. Effects of ethylene antagonist's fumigation on fruit firmness of pear cv. 'Packham's Triumph' and 'Beurre Bosc' after 4 and 6 months of cold storage.

Ethylene antagonists	Firmness (N)					
	Packham's Triumph		Mean (T)	Beurre Bosc		Mean (T)
	4 M	6 M		4 M	6 M	
Control	22.7±0.1a	25.3±0.9ab	23.9a	31.9±0.9a	29.9±0.4a	30.9a
1-MCP	69.3±0.6g	59.9±0.5f	64.7d	73.2±1.0e	48.1±0.7cd	60.6c
1-HCP	30.5±0.2bcd	25.8±0.4ab	28.1b	52.7±4.4d	47.4±0.6bcd	50.1b
(S)-(-)-Lim	32.9±0.6de	27.6±0.6abc	30.3b	36.1±0.7ab	35.6±0.5a	35.8a
TCA	36.0±0.9e	32.1±0.9cde	34.1c	38.8±0.6abc	32.9±0.2a	35.8a
Mean (SP)	38.3b	34.2a		46.5b	38.8a	
LSD ( $P \leq 0.05$ )	T = 3.4	SP = 2.2	T × SP = 4.8	T = 7.7	SP = 4.9	T × SP = 10.9

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M), T= Treatments, SP = Storage period, NS = Non-significant.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

**6.3.4. TA, SSC, SSC/TA ratio**

The effects of ethylene antagonists and cold storage periods on TA, SSC and SSC/TA ratio in both pear cultivars are presented in Table 6.6 and 6.7. Mean TA of 'Packham's Triumph' pear fruit was significantly higher when fumigated with 1-MCP (0.36 %) and (*S*)-(-)-limonene (0.35 %) as compared to all other fumigation treatments and control (Table 6.6). After 4 months CS, the mean TA was found to be significantly higher (0.39 %) than 6 months cold stored fruit (0.20 %). The interaction between treatments of ethylene antagonists and cold storage periods was not significant for TA in 'Packham's Triumph' pear (Table 6.6). In 6 months cold stored fruit, significantly higher mean SSC was recorded (13.35 %) than those stored for 4 months (4.95 %). However, effects of ethylene antagonists and their interaction with cold storage periods were found to be non-significant for SSC in 'Packham's Triumph' pear. Mean SSC/TA ratio was significantly higher in control fruit (54.7) followed by TCA fumigated fruit (45.4) in comparison to all other fumigation treatments. Mean SSC/TA ratio was significantly higher in 6 months cold stored fruit (70.3) than 4 months (12.8). The interaction between ethylene antagonists and cold storage periods was found to be significant for SSC/TA ratio in 'Packham's Triumph'. After 4 months CS, there was no significant difference among fumigation treatments and control fruit while after 6 months CS, significantly higher SSC/TA ratio was recorded in control fruit (94.3) as compared to those fumigated with different ethylene antagonists (Table 6.6).

In 'Beurré Bosc' pear, 1-MCP fumigated fruit exhibited a significantly high mean TA (0.37 %) as compared to all other fumigation treatments and control (Table 6.7). Nevertheless, 1-HCP, (*S*)-(-)-limonene and TCA fumigated fruit also maintained a significantly higher mean TA (0.24 - 0.29 %) than control (0.15 %). Mean TA was significantly higher after 4 months CS (0.31 %) when compared to 6 months cold stored fruit (0.21 %). The interaction of ethylene antagonist treatments and cold storage periods was significant for TA. Mean SSC was significantly higher in control fruit (14.9 %) followed by 1-HCP (14.6 %) and TCA fumigated fruit (14.5 %) in comparison to other ethylene antagonists. Mean SSC was significantly high after 4 months CS (15.1 %) than 6 months (13.9 %). A significant interaction was observed between ethylene antagonists and cold storage periods for SSC in 'Beurré Bosc' pear. Significantly increased SSC value was recorded in control fruit (15.93 %) after 4

months CS as compared to all other fumigation treatments. Control 'Beurré Bosc' fruit also exhibited significantly higher mean SSC/TA ratio (116.0) as compared to those fumigated with ethylene antagonists (45.4 - 61.6). There was a non-significant interaction between ethylene antagonist treatments and cold storage periods for SSC/TA ratio in 'Beurré Bosc' pear fruit (Table 6.7).

Table 6.6. Effects of ethylene antagonist's fumigation on TA, SSC and SSC/TA ratio of pear cv. 'Packham's Triumph' after 4 and 6 months of cold storage.

Ethylene antagonists	TA (%)		Mean (T)	SSC (%)		Mean (T)	SSC/TA ratio		Mean (T)
	4 M	6 M		4 M	6 M		4 M	6 M	
Control	0.31±0.01	0.15±0.01	0.23a	4.67±0.04	13.67±0.06	9.17	15.1±0.3a	94.3±3.5d	54.7d
1-MCP	0.46±0.01	0.26±0.01	0.36c	5.00±0.02	13.10±0.02	9.05	10.8±0.3a	51.8±1.1b	31.2a
1-HCP	0.40±0.01	0.19±0.01	0.29b	5.30±0.19	13.50±0.02	9.40	13.3±0.6a	72.8±2.3c	43.0bc
(S)-(-)-Lim	0.46±0.01	0.25±0.02	0.35c	4.83±0.04	13.23±0.04	9.03	10.6±0.2a	55.9±3.9b	33.3ab
TCA	0.36±0.01	0.17±0.01	0.27ab	4.97±0.05	13.23±0.06	9.10	13.9±0.5a	76.9±2.5c	45.4cd
Mean (SP)	0.39b	0.20a		4.95a	13.35b		12.8a	70.3b	
LSD ( $P \leq 0.05$ )	T = 0.05 SP = 0.03 T × SP = NS			T = NS SP = 0.24 T × SP = NS			T = 10.6 SP = 6.7 T × SP = 15.1		

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M), T = Treatments, SP = Storage period, NS = Non-significant.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 6.7. Effects of ethylene antagonist's fumigation on TA, SSC and SSC/TA ratio of pear cv. 'Beurre Bosc' after 4 and 6 months of cold storage.

Ethylene antagonists	TA (%)		Mean (T)	SSC (%)		Mean (T)	SSC/TA ratio		Mean (T)
	4 M	6 M		4 M	6 M		4 M	6 M	
Control	0.17±0.01ab	0.12±0.01a	0.15a	15.93±0.04f	13.83±0.04ab	14.9d	108.5±11.1	124.1±10.2	116.0b
1-MCP	0.50±0.02e	0.24±0.01bcd	0.37c	14.37±0.05c	14.50±0.02c	14.4b	29.6±1.5	61.3±2.5	45.4a
1-HCP	0.23±0.01bc	0.25±0.01bcd	0.24b	15.27±0.05e	14.00±0.02b	14.6c	67.9±3.5	55.3±1.2	61.6a
(S)-(-)-Lim	0.33±0.01d	0.25±0.01bcd	0.29b	14.80±0.02d	13.60±0.02a	14.2a	45.2±1.7	53.7±1.1	49.5a
TCA	0.30±0.0cd	0.20±0.01ab	0.25b	15.20±0.02e	13.90±b	14.5bc	50.7±0.1	71.1±3.5	60.9a
Mean (SP)	0.31b	0.21a		15.1b	13.9a		60.4	73.1	
LSD ( $P \leq 0.05$ )	T = 0.06	SP = 0.04	T × SP = 0.09	T = 0.17	SP = 0.11	T × SP = 0.24	T = 25.4	SP = NS	T × SP = NS

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1  $\mu$ M), (S)-(-)-Lim = (S)-(-)-limonene (1  $\mu$ M), TCA = *trans*-cinnamaldehyde (1  $\mu$ M), T = Treatments, SP = Storage period, NS = Non-significant.  $\pm$  = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

**6.3.5. Total and Individual Sugars**

In 'Packham's Triumph' pear, mean sucrose concentration was significantly higher in 1-HCP treated fruit ( $11.5 \text{ g kg}^{-1}$ ) as compared to other fumigation treatments and control (Table 6.8). Mean sucrose concentration was found to be significantly higher in 4 months cold stored fruit ( $8.93 \text{ g kg}^{-1}$ ) than 6 months ( $6.06 \text{ g kg}^{-1}$ ). The interaction between ethylene antagonists and cold storage periods was found to be significant for the levels of sucrose in 'Packham's Triumph' pear fruit. 1-HCP fumigated fruit after 4 months CS exhibited significantly higher levels of sucrose ( $16.7 \text{ g kg}^{-1}$ ) in comparison to all other fumigation treatments and control. Mean glucose concentration was found to be considerably high in (*S*)-(-)-limonene fumigation treatment ( $9.5 \text{ g kg}^{-1}$ ) followed by control fruit ( $9.3 \text{ g kg}^{-1}$ ) as compared to all other ethylene antagonist treatments. The interaction between ethylene antagonists and cold storage periods was found to be significant for glucose level in 'Packham's Triumph'. A relatively increased concentration of glucose was noted in (*S*)-(-)-limonene fumigated fruit ( $10.9 \text{ g kg}^{-1}$ ) and control ( $10.4 \text{ g kg}^{-1}$ ) after 4 months CS and in 1-MCP fumigated fruit ( $8.9 \text{ g kg}^{-1}$ ) after 6 months CS. Effect of fumigation treatments and their interaction with cold storage periods was non-significant for fructose concentration in 'Packham's Triumph' pear fruit. However, significantly higher mean concentration of fructose was noted after 4 months CS ( $150.5 \text{ g kg}^{-1}$ ) than 6 months ( $75.5 \text{ g kg}^{-1}$ ). Mean concentration of total sugars was significantly higher in 4 months cold stored fruit ( $167.4 \text{ g kg}^{-1}$ ) than 6 months ( $89.6 \text{ g kg}^{-1}$ ). Interaction of ethylene antagonists and cold storage periods was found to be significant for total sugars. 1-HCP fumigated 'Packham's Triumph' fruit exhibited significantly higher concentration of total sugars ( $199.2 \text{ g kg}^{-1}$ ) after 4 months CS as compared to all other fumigation treatments and control (Table 6.8).

Mean sucrose concentrations in 'Beurré Bosc' pear were significantly higher in 1-HCP ( $6.63 \text{ g kg}^{-1}$ ) and TCA ( $7.45 \text{ g kg}^{-1}$ ) fumigated fruit as compared to all other fumigation treatments of ethylene antagonists and control (Table 6.9). Four months cold stored fruit exhibited significantly higher mean sucrose level ( $6.17 \text{ g kg}^{-1}$ ) than 6 months CS ( $4.65 \text{ g kg}^{-1}$ ). Mean glucose concentrations were significantly higher in TCA ( $10.79 \text{ g kg}^{-1}$ ), (*S*)-(-)-limonene ( $10.70 \text{ g kg}^{-1}$ ) fumigated and control fruit ( $10.45 \text{ g kg}^{-1}$ ) as compared to those fumigated with 1-MCP and 1-HCP. The interaction of

ethylene antagonists and cold storage periods was found to be significant for glucose concentration in 'Beurré Bosc'. An elevated concentration of glucose was noted in the control fruit ( $11.31 \text{ g kg}^{-1}$ ) after 4 months CS, while after 6 months CS, it was higher in TCA fumigated fruit ( $11.25 \text{ g kg}^{-1}$ ) in comparison to all other fumigation treatments. TCA fumigated fruit showed significantly higher mean fructose concentration ( $120.9 \text{ g kg}^{-1}$ ) as compared to all other fumigation treatments and control. The six months cold stored fruit exhibited significantly higher mean fructose concentration ( $128.9 \text{ g kg}^{-1}$ ) than 4 months CS ( $87.1 \text{ g kg}^{-1}$ ). The interaction of ethylene antagonists and cold storage periods was found to be significant for fructose concentration of 'Beurre Bosc'. Relatively higher concentrations of fructose were observed in 1-HCP and TCA fumigated and control fruit ( $142.5$ ,  $133.5$  and  $132.5 \text{ g kg}^{-1}$  respectively) after 6 months CS in comparison to all other fumigation treatments. The levels of mean total sugars were significantly increased in control ( $124.5 \text{ g kg}^{-1}$ ), 1-HCP ( $123.2 \text{ g kg}^{-1}$ ) and (*S*)-(-)-limonene ( $120.8 \text{ g kg}^{-1}$ ) fumigated fruit in comparison to 1-MCP and TCA fumigation treatments. The mean concentration of total sugars was significantly higher after 6 months CS ( $143.6 \text{ g kg}^{-1}$ ) than 4 months ( $103.1 \text{ g kg}^{-1}$ ). The interaction of ethylene antagonists and cold storage periods was significant for total sugars of 'Beurré Bosc' pear fruit. Slightly highest concentration of total sugars was found in 1-HCP and (*S*)-(-)-limonene fumigated fruit ( $156.5$  and  $151.3 \text{ g kg}^{-1}$  respectively) after 6 months CS as compared to all other fumigation treatments and control (Table 6.9).

Table 6.8. Effects of ethylene antagonist's fumigation on individual and total sugars of pear cv. 'Packham's Triumph' after 4 and 6 months of cold storage.

Ethylene antagonists	Sugars (g kg <sup>-1</sup> )											
	Sucrose		Mean (T)	Glucose		Mean (T)	Fructose		Mean (T)	Total sugars		Mean (T)
	4 M	6 M		4 M	6 M		4 M	6 M		4 M	6 M	
Control	5.7±0.4ab	4.8±0.0a	5.3a	10.4±0.1d	8.0±0bc	9.3cd	149.7±0.7	67.4±3.4	108.6	165.8±1.1b	80.3±0.6a	123.0
1-MCP	7.3±0.4b	4.5±0.0a	6.1ab	6.9±0abc	8.9±0cd	8.1bc	149.9±0.9	69.6±2.3	109.8	164.2±1.4b	83.5±2.3a	123.8
1-HCP	16.7±0.5c	6.3±0.0ab	11.5d	5.5±0.1a	7.4±0abc	6.5a	177.1±0.7	67.3±2.1	122.2	199.2±1.0c	81.0±2.1a	140.1
(S)-(-)-Lim	7.7±0.3b	6.2±0.0ab	6.9bc	10.9±0.8d	8.0±0bc	9.5d	134.3±12.6	89.5±1.6	111.9	152.9±12.1b	103.8±1.6a	128.3
TCA	7.4±0.4b	8.1±0.0b	7.7c	6.1±0.1ab	7.4±0abc	6.7ab	141.7±0.5	83.7±3.0	112.7	155.1±0.3b	99.2±3.0a	127.1
Mean (SP)	8.93b	6.06a		7.97	7.98		150.5b	75.5a		167.4b	89.6a	
LSD ( $P \leq 0.05$ )	T = 1.52 SP = 0.96 T×SP = 2.15			T = 1.3 SP = NS T×SP = 1.9			T = NS SP = 14.42 T×SP = NS			T = NS	SP = 13.8	T×SP = 30.9

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = Treatments, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 6.9. Effects of ethylene antagonist's fumigation on individual and total sugars of pear cv. 'Beurre Bosc' after 4 and 6 months of cold storage.

Ethylene antagonists	Sugars (g kg <sup>-1</sup> )											
	Sucrose		Mean (T)	Glucose		Mean (T)	Fructose		Mean (T)	Total sugars		Mean (T)
	4 M	6 M		4 M	6 M		4 M	6 M		4 M	6 M	
Control	4.3±0.1	3.4±0.1	3.89a	11.3±0.3e	9.6±0.1bc	10.45c	87.8±2.6bc	132.5±0.2fg	110.2b	103.5±2.7b <sup>c</sup>	145.5±0.2ef	124.5b
1-MCP	5.9±0.4	8.4±0.1	5.11a	9.1±0.1bc	9.8±0.1bcd	9.47b	70.2±1.2a	118.4±2.2ef	94.3a	85.2±0.8a	132.6±2.2de	108.9a
1-HCP	7.9±0.5	5.4±0.1	6.63b	7.7±0.2a	8.7±0.1ab	8.17a	74.3±0.5ab	142.5±2.1g	108.4b	89.9±0.8ab	156.5±2.1f	123.2b
(S)-(-)-Lim	4.4±0.1	3.6±0.2	3.98a	10.4±0.0cde	10.9±0.1de	10.70c	94.9±3.9cd	117.4±1.2ef	106.2b	109.7±4.0c	132.0±1.1de	120.8b
TCA	6.5±0.1	4.3±0.1	7.45b	10.3±0.3cde	11.3±0.1e	10.79c	108±2.3de	133.5±0.8fg	120.9c	127.0±2.0d	151.3±0.6f	139.2c
Mean (SP)	6.17b	4.65a		9.78a	10.06b		87.1a	128.9b		103.1b	143.6a	
LSD ( $P \leq 0.05$ )	T = 1.3	SP = 0.8	T×SP = NS	T = 0.9	SP = NS	T×SP = 1.3	T = 10.7	SP = 6.7	T×SP = 15.1	T = 10.5	SP = 6.6	T×SP = 14.8

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = Treatments, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

**6.3.6. Total and Individual Organic Acids**

In 'Packham's Triumph' pear, the effect of ethylene antagonists and cold storage periods was non-significant for malic acid, succinic acid, fumaric acid and total organic acids (Table 6.10). The interaction of ethylene antagonists and cold storage periods was found to be significant for malic acid concentration in 'Packham's Triumph'. Relatively higher concentration of malic acid was noted in control fruit ( $2.87 \text{ g kg}^{-1}$ ) after 6 months CS in comparison to fumigation treatments. However, 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA fumigated fruit also showed slightly high concentration of malic acid depending upon cold storage periods. The interaction of ethylene antagonists and cold storage periods was also found to be significant for total organic acids in 'Packham's Triumph' fruit. Comparatively increased concentration of total organic acids was recorded in 6 months cold stored 'control' fruit ( $3.48 \text{ g kg}^{-1}$ ) than those fumigated with ethylene antagonists. Nevertheless, after 4 month CS, 1-MCP, (*S*)-(-)-limonene and TCA fumigated fruit exhibited moderately higher concentration of total organic acids ( $2.75$ ,  $2.99$  and  $2.81 \text{ g kg}^{-1}$  respectively) than control. While after 6 months CS, high concentration of total organic acids was recorded in 1-MCP and 1-HCP fumigated fruit ( $2.41$  and  $2.81 \text{ g kg}^{-1}$  respectively) as compared to all other fumigation treatments (Table 6.10).

In 'Beurre Bosc' pear, mean concentration of succinic acid was moderately high in (*S*)-(-)-limonene fumigation treatment ( $0.814 \text{ g kg}^{-1}$ ) followed by TCA fumigated ( $0.739 \text{ g kg}^{-1}$ ) and control fruit ( $0.452 \text{ g kg}^{-1}$ ) (Table 6.11). Mean concentration of fumaric acid was significantly high in (*S*)-(-)-limonene fumigated fruit ( $0.152 \text{ g kg}^{-1}$ ) as compared to all other fumigation treatments and control. Mean fumaric acid concentration was found significantly high in 6 months cold stored fruit ( $0.111 \text{ g kg}^{-1}$ ) than 4 months CS ( $0.081 \text{ g kg}^{-1}$ ). The interaction of ethylene antagonists and cold storage periods was found to be significant for fumaric acid concentration in 'Beurre Bosc' pear. Significantly high concentration of fumaric acid was found in (*S*)-(-)-limonene fumigated fruit after 6 months CS ( $0.22 \text{ g kg}^{-1}$ ) in comparison to all other fumigation treatments and control. Mean concentration of total organic acids was found to be considerably high in TCA fumigation treatment ( $2.413 \text{ g kg}^{-1}$ ) followed by (*S*)-(-)-limonene ( $1.864 \text{ g kg}^{-1}$ ) as compared to all other fumigation treatments and control (Table 6.11).

Table 6.10. Effects of ethylene antagonist's fumigation on individual and total organic acids of pear cv. 'Packham's Triumph' after 4 and 6 months of cold storage.

Ethylene antagonists	Organic acids (g kg <sup>-1</sup> )											
	Malic acid		Mean (T)	Succinic acid		Mean (T)	Fumaric acid		Mean (T)	Total organic acids		Mean (T)
	4 M	6 M		4 M	6 M		4 M	6 M		4 M	6 M	
Control	1.03±0.1ab	2.87±0.2d	1.952	0.32±0.1	0.54±0.0	0.43	0.083±0.0	0.077±0.0	0.080	1.44±0.1ab	3.48±0.2d	2.46
1-MCP	2.0±0.0abcd	1.78±0.1abcd	1.923	0.61±0.0	0.50±0.1	0.55	0.086±0.0	0.127±0.0	0.107	2.75±0.0bcd	2.41±0.1abcd	2.59
1-HCP	0.79±0.2a	2.27±0.2bcd	1.530	0.31±0.0	0.46±0.1	0.39	0.080±0.0	0.080±0.0	0.080	1.18±0.2a	2.81±0.3bcd	1.99
(S)-(-)-Lim	2.55±0.2cd	1.43±0.2abc	1.992	0.35±0.0	0.48±0.0	0.42	0.086±0.0	0.076±0.0	0.082	2.99±0.2cd	1.99±0.2abc	2.49
TCA	2.45±0.3bcd	1.38±0.2abc	1.918	0.27±0.0	0.34±0.0	0.31	0.090±0.0	0.086±0.0	0.088	2.81±0.3bcd	1.82±0.2abc	2.32
Mean (SP)	1.78	1.95		0.373	0.465		0.085	0.089		2.24	2.50	
LSD ( $P \leq 0.05$ )	T = NS SP = NS T×SP = 1.26			T = NS SP = NS T×SP = NS			T = NS SP = NS T×SP = NS			T = NS SP = NS T×SP = 1.305		

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = Treatments, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 6.11. Effects of ethylene antagonist's fumigation on individual and total organic acids of pear cv. 'Beurre Bosc' after 4 and 6 months of cold storage

Ethylene antagonists	Organic acids (g kg <sup>-1</sup> )											
	Malic acid		Mean (T)	Succinic acid		Mean (T)	Fumaric acid		Mean (T)	Total organic acids		Mean (T)
	4 M	6 M		4 M	6 M		4 M	6 M		4 M	6 M	
Control	0.69±0.1	0.43±0.0	0.56	0.56±0.1	0.34±0.0	0.452abc	0.082±0.0a	0.083±0.0a	0.082a	1.35±0.1ab	0.85±0.1a	1.098a
1-MCP	0.58±0.1	1.34±0.2	0.96	0.27±0.0	0.22±0.0	0.243a	0.076±0.0a	0.078±0.0a	0.077a	0.93±0.1a	1.64±0.2ab	1.286a
1-HCP	1.24±0.2	0.82±0.1	1.03	0.38±0.0	0.26±0.0	0.320ab	0.082±0.0a	0.087±0.0a	0.085a	1.69±0.2ab	2.54±0.1b	1.433a
(S)-(-)-Lim	1.43±0.2	1.47±0.2	1.45	0.77±0.1	0.86±0.2	0.814c	0.083±0.0a	0.22±0.0b	0.152b	2.28±0.1ab	1.17±0.4ab	1.864a
TCA	1.19±0.2	0.86±0.1	1.04	0.85±0.0	0.63±0.2	0.739bc	0.082±0.0a	0.088±0.0a	0.086a	2.13±0.2ab	1.60±0.2ab	2.413b
Mean (SP)	1.03	0.99		0.567	0.461		0.0813b	0.1115a		1.677	1.561	
LSD ( $P \leq 0.05$ )	T = NS SP = NS T×SP = NS			T = 0.43 SP = NS T×SP = NS			T = 0.01 SP = 0.005 T×SP = 0.01			T = 0.90 SP = NS T×SP = NS		

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = Treatments, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

**6.3.7. Ascorbic Acid**

Mean concentration of ascorbic acid in 'Packham's Triumph' pear was relatively high in 1-MCP fumigated fruit (54.0 mg Kg<sup>-1</sup>) followed by (*S*)-(-)-limonene (43.8 mg Kg<sup>-1</sup>) in comparison to all other fumigation treatments and control (Table 6.12). Mean concentration of ascorbic acid was also comparatively higher in 1-HCP and TCA fumigated fruit (30.7 and 41.8 mg Kg<sup>-1</sup> respectively) than control fruit (18.4 mg Kg<sup>-1</sup>). The effect of cold storage periods and their interaction with ethylene antagonists was non-significant for ascorbic acid in 'Packham's Triumph' pear fruit (Table 6.12).

In 'Beurre Bosc' pear, mean concentration of ascorbic acid was found to be significantly higher in 1-MCP, 1-HCP and TCA fumigated fruit (22.8, 19.3 and 20.6 mg Kg<sup>-1</sup> respectively) as compared to those treated with the (*S*)-(-)-limonene (14.5 mg Kg<sup>-1</sup>) and control (9.9 mg Kg<sup>-1</sup>). Mean ascorbic acid level was significantly higher after 4 months CS (20.6 mg Kg<sup>-1</sup>) than 6 months (14.2 mg Kg<sup>-1</sup>). The interaction of fumigation treatments and cold storage periods was found to be non-significant for ascorbic acid concentration in 'Beurre Bosc' pear fruit (Table 6.13).

**6.3.8. Total Antioxidants**

Mean total antioxidants of 'Packham's Triumph' pear fruit were significantly higher in (*S*)-(-)-limonene (43.37 mM Trolox kg<sup>-1</sup>) and TCA fumigated fruit (40.62 mM Trolox kg<sup>-1</sup>) as compared to all other fumigation treatments and control. The higher mean total antioxidants were recorded after 4 months cold storage (38.11 mM Trolox kg<sup>-1</sup>) than 6 months cold stored fruit (27.40 mM Trolox kg<sup>-1</sup>). The interaction of ethylene antagonists and cold storage periods was found to be significant for total antioxidants in 'Packham's Triumph'. After 4 months CS, significantly higher antioxidant activity was recorded in (*S*)-(-)-limonene (56.96 mM Trolox kg<sup>-1</sup>) and TCA (55.68 mM Trolox kg<sup>-1</sup>) fumigated fruit; while 1-MCP fumigated fruit exhibited significantly increased total antioxidants (37.69 mM Trolox kg<sup>-1</sup>) after 6 months CS (Table 6.12).

'Beurre Bosc' pear fruit showed significantly higher mean total antioxidants when fumigated with (*S*)-(-)-limonene (21.11 mM Trolox kg<sup>-1</sup>) and TCA (15.99 mM Trolox kg<sup>-1</sup>) as compared to all other treatments of ethylene antagonists and control.

The effect of cold storage periods was non-significant for antioxidant activity in 'Beurre Bosc'. Moreover, the interaction of ethylene antagonists and cold storage periods was also non-significant for total antioxidant in 'Beurre Bosc' (Table 6.13).

Table 6.12. Effects of ethylene antagonist's fumigation on ascorbic acid and total antioxidants of pear cv. 'Packham's Triumph' after 4 and 6 months of cold storage.

Ethylene antagonists	Ascorbic acid (mg Kg <sup>-1</sup> )		Mean (T)	Total antioxidants (mM Trolox Kg <sup>-1</sup> )		Mean (T)
	4 M	6 M		4 M	6 M	
Control	18.9±1.1	17.8±1.0	18.4a	19.06±0.3a	19.77±0.4a	19.41a
1-MCP	57.5±4.9	50.6±2.0	54.0d	26.79±0.8abc	37.69±0.5d	32.24b
1-HCP	32.7±1.1	28.8±1.0	30.7b	32.05±1.6cd	24.20±0.6ab	28.13b
(S)-(-)-Lim	46.5±2.0	41.2±0.5	43.8cd	56.96±1.1e	29.78±0.4bc	43.37c
TCA	43.7±4.1	39.8±1.8	41.8bc	55.68±2.0e	25.55±0.3abc	40.62c
Mean (SP)	39.8	35.6		38.11b	27.40a	
LSD ( $P \leq 0.05$ )	T = 11.2	SP = NS	T × SP = NS	T = 4.97	SP = 3.14	T × SP = 7.02

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = Treatments, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

Table 6.13. Effects of ethylene antagonist's fumigation on ascorbic acid and total antioxidants of pear cv. 'Beurre Bosc' after 4 and 6 months of cold storage.

Ethylene antagonists	Ascorbic acid (mg Kg <sup>-1</sup> )		Mean (T)	Total antioxidants (mM Trolox kg <sup>-1</sup> )		Mean (T)
	4 M	6 M		4 M	6 M	
Control	12.29±1.1	7.60±0.7	9.9a	10.58±0.2	9.05±0.4	9.82a
1-MCP	24.79±0.7	20.83±0.9	22.8c	12.94±0.1	11.11±0.1	12.03a
1-HCP	24.13±0.5	14.49±0.3	19.3c	12.99±0.1	10.83±0.1	11.91a
(S)-(-)-Lim	15.04±0.5	13.94±0.7	14.5b	21.22±1.7	20.99±0.2	21.11c
TCA	26.89±0.6	14.21±1.8	20.6c	16.47±0.7	15.51±0.3	15.99b
Mean (SP)	20.63b	14.22a		14.84	13.50	
LSD ( $P \leq 0.05$ )	T = 4.2	SP = 2.7	T × SP = NS	T = 3.1	SP = NS	T × SP = NS

4 M = 4 months cold storage, 6 M = 6 months cold storage, 1-MCP = 1-methylcyclopropene (740 ppb), 1-HCP = 1-hexylcyclopropene (1 µM), (S)-(-)-Lim = (S)-(-)-limonene (1 µM), TCA = *trans*-cinnamaldehyde (1 µM), T = Treatments, SP = Storage period, NS = Non-significant. ± = Standard error of the mean (SE). Means followed by the same letter in columns and rows are not significantly different at  $P \leq 0.05$ .

#### **6.4. Discussion**

Climacteric fruits are characterized by exhibiting an exponential increase in ethylene production which correlates with fruit ripening (Huber, 2008). This burst in rate of climacteric ethylene production leads to rapid decline of fruit quality and shortens storage life of pear fruit (Bower *et al.*, 2003; Gapper *et al.*, 2006). Fumigation with different ethylene antagonists and cold storage periods have significantly influenced the onset of ethylene climacteric peak and the rate of climacteric ethylene production. 1-MCP fumigation was highly effective in suppressing the production rate of climacteric ethylene peaks and also delayed the onset of ethylene peaks in both pear cultivars as compared to other ethylene antagonists and control (Table 6.1 and 6.2). The highly reduced rate of ethylene production in both pear cultivars with 1-MCP fumigation may possibly be ascribed to the stabilization of ethylene receptor proteins by 1-MCP which consequently inhibit ethylene production and its action on fruit ripening (Kevany *et al.*, 2007 and 2008). Similarly, decreased rate of ethylene production have also been reported earlier in ‘Blanquilla’ and ‘Bartlett’ pear with 1-MCP application (Larrigaudiere *et al.*, 2004; Ekman *et al.*, 2004). Further, Trinchero *et al.* (2004) and Gapper *et al.* (2006) reported declined ethylene production in ‘Bartlett’ and ‘d’Anjou’ pear respectively with 1-MCP treatment after cold storage.

In ‘Packham’s Triumph’ pear, 1-HCP fumigation also suppressed ethylene production at climacteric peak while in ‘Beurré Bosc’, (*S*)-(-)-limonene fumigation led to reduced rate of climacteric ethylene peak following 1-MCP fumigation treatment depending upon cold storage period. (*S*)-(-)-limonene and TCA fumigation treatments also played an effective role in delaying the onset of ethylene climacteric peaks in ‘Packham’s Triumph’ and ‘Beurré Bosc’ pear (Table 6.1 and 6.2). According to Sisler *et al.* (2003), suppression in ethylene production by 1-HCP treatment is probably due to its binding with ethylene receptors similar to 1-MCP. Fumigation of 1-HCP has also been reported earlier to delay and suppress the climacteric ethylene production during fruit ripening in ‘Kommeet’ tomato (Khan *et al.*, 2016). Both 1-MCP and 1-HCP are cyclopropenes with 1-substituted chains and are hydrophobic in nature which increase their affinity with ethylene receptors (Sisler *et al.*, 2003) and makes them effective to inhibit ethylene action. A glance over the structure of TCA reveals its potential for antagonism as it contains oxygen with double bond which increases its potency by

increasing hydrophobicity. In addition, the oxygen atom withdraws electrons from its double bond and can help to make a strong bond with receptor sites (Sisler *et al.*, 2006). However, there are no previous reports on the effects of (*S*)-(-)-limonene and TCA on climacteric ethylene production during fruit ripening but has been reported to inhibit flower abscission induced by ethylene in waxflower (Abdalghani, 2017). Ethylene production rate in control ‘Packham’s Triumph’ and ‘Beurre Bosc’ pear increased sharply as compared to fumigated fruit, when shifted from cold room to simulated shelf conditions. Possibly, it may be attributed to the chilling temperatures during cold storage which act as a stimulus to induce and upregulate biosynthesis of key enzymes involved in ethylene biosynthesis such as ACC synthase and ACC oxidase enzymes (Villalobos-Acuna *et al.*, 2011). The rate of climacteric ethylene production was reduced in 6 months cold stored pear fruit than those stored for 4 months while onset of ethylene climacteric peak was not influenced by cold storage periods. Reduced ethylene production in 6 months cold stored fruit in both pear cultivars than in 4 months can be explained by the fact that in pears ethylene peaks approach earlier with higher magnitudes and with an extension of cold storage periods, rate of climacteric ethylene declines. This phenomenon has also been reported earlier in ‘Doyenne du Comice’ pear after 4, 5 and 6 months of cold storage (Wang and Sugar, 2013).

Parameters of respiratory climacteric in ‘Packham’s Triumph’ and ‘Beurre Bosc’ pear were also affected by ethylene antagonists and cold storage periods with some exceptions. To illustrate, onset of respiratory climacteric peak and rate of respiration at climacteric was significantly delayed and suppressed by 1-MCP fumigation as compared to all other fumigation treatments and control (Table 6.3 and 6.4). Previously, application of 1-MCP has also been reported to reduce respiration rate in ‘Bartlett’ and d’Anjou’ pear (Trincherro *et al.* 2004; Gapper *et al.* 2006). In addition, 1-HCP fumigation also had a positive effect in delaying and reducing the climacteric respiration peak in both the tested pear cultivars. Similarly, 1-HCP treated tomato fruit exhibited delayed and reduced climacteric respiration peak as compared to the control (Khan *et al.*, 2016). Moreover, (*S*)-(-)-limonene and TCA fumigation also effectively reduced the respiration rate and delayed the respiratory climacteric peak in comparison to the control (Table 6.3 and 6.4). Reduced respiration rate by the fumigation treatments could be due to the suppressed ethylene production by these

treatments as de Wild *et al.* (1999) claimed that there was inhibition of ethylene production and respiration rate in ‘Conference’ pear at similar levels by CO<sub>2</sub> as an inhibitor of ethylene action. It can also be argued otherwise that reduced respiration rate with the fumigation of different ethylene antagonists is probably responsible for reduced rate of ethylene production in pear cultivars. Previously, Apelbaum *et al.* (1981) reported a reduction of respiration rate resulting in low rates of ATP production which is required to activate ACC oxidase.

Market quality of pear fruit highly depends on its firmness (Trincherro *et al.*, 2004). Fruit firmness of both cultivars was effectively retained in 4 and 6 months cold stored fruit which were fumigated with 1-MCP (Table 6.5). Spotts *et al.* (2007) also claimed that 1-MCP treatment resulted in higher flesh firmness in ‘d’Anjou’ pear after 8 months of cold storage. Similarly, 1-MCP treatment inhibited loss of firmness in ‘La France’ pear cultivar after 3 weeks of cold storage (Hiwasa *et al.*, 2003) and in ‘Bartlett’ pear after 30 days of cold storage (Trincherro *et al.*, 2004) and during subsequent shelf life. Loss of firmness was also inhibited by TCA in ‘Packham’s Triumph’ and with 1-HCP in ‘Beurre Bosc’ pear. Loss of fruit firmness during ripening mainly occurs by the modification of the primary cell wall due to an increased activity of cell wall hydrolases. Ethylene plays an important role in these processes of pear fruit softening (Hiwasa *et al.*, 2003). 1-MCP treatment was found to inhibit the activity of  $\beta$ -galactosidase enzyme and suppressed the accumulation of transcripts for *PpGAL* genes in ‘LaFrance’ pear (Mwaniki *et al.*, 2005). Higher fruit firmness with 1-HCP and TCA following 1-MCP in the present study may be attributed to decreased activity of fruit softening enzymes by these fumigation treatments. Moreover, as ethylene plays an important role in the process of fruit softening (Hiwasa *et al.*, 2003), fruit firmness retention in both pear cultivars fumigated with 1-MCP, 1-HCP and TCA attributed to suppressed ethylene production with these treatments. Johnston *et al.* (2009) also reported that fruit softening which is associated with ripening events is highly dependent on ethylene. Both pear cultivars soften normally at simulated shelf conditions after both cold storage periods.

In control fruit of ‘Packham’s Triumph’ and ‘Beurré Bosc’ pear, firmness declined remarkably during cold storage and decreased even sharply after shifting to simulated shelf conditions as compared to fruit which received fumigation of ethylene antagonists. In both pear cultivars, the fruit firmness declined after long term cold

storage of 6 months in comparison to 4 months CS. It is probably because after a finite time span following treatment with ethylene antagonists, fruit tissues may have again become prone to ethylene either due to the formation of new binding sites on receptors or by the dissociation of sites from ethylene inhibitors (Feng *et al.*, 2004).

TA of both pear cultivars was significantly high with 1-MCP fumigation treatment followed by (*S*)-(-)-limonene in ‘Packham’s Triumph’ fruit. Nonetheless, 1-HCP and TCA also maintained relatively higher TA in both cultivars as compared to the control (Table 6.6 and 6.7). Moreover, with increasing cold storage period, a reduction in TA was recorded. Malic acid, which is a predominant organic acid in pear fruit is used in tri-carboxylic acid cycle during the process of respiration and leads to reduced TA. Comparatively higher TA with ethylene antagonists is probably due to reduced respiration rate with the fumigation treatments, ultimately inhibiting the loss of TA (Chen *et al.*, 1982; Wang and Sugar, 2013).

Changes in the levels of sugars and organic acids in the fruit can affect their organoleptic features and overall fruit quality (Arzani *et al.*, 2008). The levels of sugars and organic acids in the fruit vary among genotypes, fruit maturity, storage conditions and cultural practices (Teng and Liu, 1999). Hence, maintenance of sugars and organic acids is very important to prevent the loss of fruit taste (Itai and Tanahashi, 2008). Individual and total sugars of ‘Packham’s Triumph’ and ‘Beurré Bosc’ varied among fumigation treatments and the control fruit as well as between 4 and 6 months cold storage periods. However, no pronounced effect of ethylene antagonists and cold storage periods was noted (Table 6.8 and 6.9). In general, fructose concentration was higher than sucrose and glucose in both pears cultivars. Higher differences in fructose level between ‘Packham’s Triumph’ and ‘Beurré Bosc’ pear after 4 and 6 months CS can be ascribed to the varying activity of sugar degrading and synthesizing enzymes (Yamada *et al.*, 2006). Considerably lower sucrose concentration in both pear cultivars after 4 and 6 months of cold storage than glucose and fructose is consistent with the findings of Itai and Tanahasi (2008) who claimed extremely low concentration of sucrose in cultivars of Japanese plum after 4 weeks cold storage. It can be surmised from previous studies that loss of sucrose is not the result of cold storage, rather it occurs during simulated shelf storage (Ding *et al.*, 1998; Mao *et al.*, 2006).

For individual and total organic acids, the effects of fumigation treatments and cold storage periods were non-significant in 'Packham's Triumph' while in 'Beurré Bosc', (*S*)-(-)-limonene and TCA fumigation treatments resulted in slightly higher concentration of total organic acids than all other ethylene antagonists and control (Table 6.10 and 6.11). Moreover, in 'Beurré Bosc' concentration of malic acid, succinic acid and total organic acids decreased after long term storage of 6 months than 4 months. It could be due to the utilization of these organic acids in the biosynthesis of esters or as a precursor of respiration (Arzani *et al.*, 2008).

Antioxidant capacity of fruit is one of the important health benefiting compounds and is responsible for providing protection from AOS (active oxygen species) which are normally produced during stress conditions. In pear fruit, cold storage results in the generation of AOS and leads to various storage disorders, hence, antioxidant activity of pears is an indication of their potential to fight oxidative stress (Larrigaudiere *et al.*, 2004). Antioxidant activity of 'Packham's Triumph' and 'Beurré Bosc' pear was significantly high with (*S*)-(-)-limonene and TCA fumigation treatments as compared to 1-MCP, 1-HCP and control (Table 6.12 and 6.13). Increased antioxidants with compounds of (*S*)-(-)-limonene and TCA can improve the quality of pear fruit after cold storage as an increase in the antioxidant activity has been reported to slow down the process of senescence in fruit (Lacan and Baccou, 1998).

All ethylene antagonist treatments resulted in comparatively higher concentration of ascorbic acid in tested pear cultivars than control (Table 6.12 and 6.13). Higher ascorbic acid concentration in 'Packham's Triumph' and 'Beurré Bosc' pear with all tested ethylene antagonists after cold storage suggest that these compounds can help to maintain physico-chemical properties of pear fruit after prolonged storage. Ascorbic acid concentration normally declines during cold storage of pears probably due to an increase in the concentration of H<sub>2</sub>O<sub>2</sub> as a result of oxidative stress. It leads to oxidation of ascorbate as ascorbate peroxidase utilize two molecules of ascorbate and ultimately reduce H<sub>2</sub>O<sub>2</sub> to detoxify plant cells (Larrigaudiere *et al.*, 2004). A significant reduction in ascorbic acid content of control 'Doyenne du Comice' pear fruit has also been reported by Wang and Sugar (2013). Ascorbic acid content is affected greatly by cultivar as well as by storage conditions and reflects the antioxidant activity of fruit (Sanchez-Moreno *et al.*, 2003).

Comparison of genotypes reveals that ‘Packham’s Triumph’ pear fruit had relatively higher antioxidant capacity and ascorbic acid concentration than ‘Beurré Bosc’. It has been reported by Sanchez-Moreno *et al.* (2003) earlier that green pears are rich in antioxidant compounds.

Briefly, 1-MCP application has slowed down the ripening process and associated quality changes during cold storage as compared to all other ethylene antagonists and control. Cinnamaldehyde has been found to alleviate the oxidative stress in ‘Cut Rose’ by upregulating the activities of antioxidant enzymes (Jing *et al.*, 2011) which probably contribute to inhibiting the loss of fruit quality in present study. Limonene has been used as an insecticidal and antimicrobial compound in previous studies (Ibrahim *et al.*, 2004; Hollingsworth, 2005) but current results suggest that it can also be used as an effective ethylene antagonist to protect fruit from the deleterious effect of ethylene. In addition, 1-HCP also reflected high anti-ethylene activity in the current study on pear cultivars manifesting that 1-substituted cyclopropenes with long side chains have more potency as ethylene antagonist (Sisler *et al.*, 2003; Serek *et al.*, 2007; Khan *et al.*, 2016).

In conclusion, suppression and delay of ethylene and respiratory climacteric peaks by 1-MCP fumigation treatments and ultimate maintenance of fruit quality in ‘Packham’s Triumph’ and ‘Beurré Bosc’ pear is consistent with earlier studies. 1-MCP has been reported to impede ripening process in many pear cultivars (Lelièvre *et al.*, 1997; Baritelle *et al.*, 2001; Hiwasa *et al.*, 2003; Argenta *et al.*, 2003; Trincherro *et al.*, 2004; Calvo and Sozzi, 2004). However, the results of present research indicate that 1-HCP, (S)-(-)-limonene and TCA also retard the ripening of pear fruit depending upon storage periods. These are new compounds to be used as ethylene antagonists and have been shown to exhibit ethylene inhibitory effects in some previous reports (Khan *et al.*, 2016; Abdalghani, 2017).

## **CHAPTER 7**

### **General Discussion, Conclusion and Recommendations**

#### **7.1. Introduction**

Pome fruits including apple and pear are extensively consumed worldwide due to their beneficial impact on human health as they are a rich source of dietary fibre, polyphenols, antioxidants, vitamins and mineral elements (Hyson, 2011; Kou *et al.*, 2014). Marketing of fresh horticulture produce poses great challenges because of their highly perishable nature and involves huge amounts of postharvest losses of 15-50 % during the supply chain (Romanazzi *et al.*, 2017). Hence, commercial horticulture demands proper postharvest management during transportation, storage and marketing to reduce the quantitative and qualitative losses to ensure food security to an ever-increasing world population (Gogo *et al.*, 2017). Ethylene, a ripening hormone, is one of the major factors responsible for postharvest losses as it plays a pivotal role in fruit ripening and senescence by generating a complex signalling cascade (Yang *et al.*, 2013; Razzaq *et al.*, 2016). Both apple and pear are typically climacteric fruits and exhibit an exponential increase in ethylene and respiration rates during fruit ripening due to autocatalytic ethylene biosynthesis (Dandekar *et al.*, 2004; Yang *et al.*, 2013). An excessive ethylene production during climacteric ripening leads to the microbial infections, physiological disorders and reduces the shelf life (Feng *et al.*, 2004). Postharvest losses by deleterious effects of ethylene can be minimized by inhibiting ethylene biosynthesis or ethylene action (Ansari and Tuteja, 2015). Inhibition of ethylene action by ethylene antagonists provides an effective approach to protect fresh horticultural produce from both endogenous and exogenous sources of ethylene by binding to ethylene receptor sites and prevent the downstream signal transduction (Pirung *et al.*, 2008; Yang *et al.*, 2013). Ethylene antagonists, particularly, 1-methylcyclopropene (1-MCP) has been used extensively for extending the storage life and maintaining quality of apple and pear fruits (Calvo and Sozzi, 2004; Bai *et al.*, 2005; Villalobos Acuna *et al.*, 2011; Wang and Sugar, 2013; Han *et al.*, 2015; Razzaq *et al.*, 2016). Postharvest storage techniques including cold and CA are essential requirements for year-round marketing of apple and pear with maintained fruit quality (Watkins, 2002; Mahajan *et al.*, 2010); however, commercial storage is associated with

various physiological disorders in pome fruits (Argenta *et al.*, 2000). Accumulation of ethylene in storage rooms from varying sources also deteriorate the fruit quality (Janssen *et al.*, 2014). Inhibition of ethylene action with ethylene antagonists can serve as a protective shield for fruits in commercial storage.

Although 1-MCP delays fruit ripening, softening and other quality losses, it has some drawbacks such as higher cost of application, flesh browning, lack of aroma development, uneven ripening, excessively hard fruit texture and highly genotype dependent effects (Watkins and Nock, 2004; Marin *et al.*, 2009; Jung *et al.*, 2009; James *et al.*, 2010; Watkins and Nock, 2012; Khan *et al.*, 2016). All these issues are of concern to the commercial industry and demand some novel ethylene antagonists as alternatives (Xu *et al.*, 2016). 1-Hexylcyclopropene (1-HCP) is one of the structural analogues of 1-MCP with longer carbon chain which has been tested as an ethylene antagonist in scientific studies with fruitful results (Kebenei *et al.*, 2003; Serek *et al.*, 2007; Khan *et al.*, 2016). Essential oils (from orange, lemon, grapefruit, eucalyptus, clove, cinnamon etc.) have been tested in various research studies to prevent postharvest spoilage and diseases of different fruits (Chebli *et al.*, 2004; Sharma and Tripathi, 2006; Viuda-Martos *et al.*, 2008; Goni *et al.*, 2009). *trans*-Cinnamaldehyde (TCA) and (*S*)-(-)-limonene which are found in essential oils have also been used to maintain fresh produce quality, prevent decay and exhibit potential to inhibit ethylene induced flower abscission in waxflower (Xing *et al.*, 2011; Lu *et al.*, 2013b; Solgi and Ghorbanpour, 2014; Carvalho *et al.*, 2016; Abdalghani, 2017). The present research aimed to evaluate the comparative effects of 1-HCP, (*S*)-(-)-limonene, TCA and 1-MCP to extend the storage life and to maintain the quality of apple and pear cultivars in combination with storage conditions and storage periods. Specific objectives of the present research project were to evaluate the effects of these compounds on ethylene production, respiration rate and fruit quality parameters of ‘Fuji’ and ‘Cripps Pink’ apple under cold and CA storage conditions. Moreover, the impact of these ethylene antagonists was also assessed on the climacteric ethylene production, respiration rate and cold storage life and quality of ‘Packham’s Triumph’ and ‘Beurre Bosc’ pear fruit.

### **7.2. Efficacy of Ethylene Antagonists in Extending Cold Storage Life and Maintaining Fruit Quality of Apple cv. ‘Fuji’ and ‘Cripps Pink’**

In two independent experiments, ‘Fuji’ and ‘Cripps Pink’ apple fruit were fumigated with 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA to investigate the effects of these different ethylene antagonists on climacteric ethylene production, respiration rate, cold storage life and fruit quality. All fumigation treatments with ethylene antagonists suppressed the rate of ethylene production and delayed the onset of climacteric ethylene peaks in ‘Fuji’ and ‘Cripps Pink’ in comparison to control after various CS periods (Table 4.1 and 4.2). However, the effect of 1-MCP was more evident in suppressing and delaying the ethylene climacteric peaks as compared to all other ethylene antagonists and control. Reduced ethylene production has been reported in different apple cultivars by the application of 1-MCP (Watkins, 2008; Lee *et al.*, 2012; Lu *et al.*, 2013a). The probable reason behind the efficacy of 1-MCP is the downregulation of ACS and ACO enzyme activities which play a key role in the biosynthesis of ethylene (Bulens *et al.*, 2011). Both 1-MCP and 1-HCP belong to the class of cyclopropenes which are highly strained compounds and their potency depend upon ring strain of their structure. Moreover, these compounds have been found to be effective in earlier studies only by single exposure as compared to the other previously used ethylene antagonists (Sisler *et al.*, 2006). The reduced climacteric ethylene production with the application of 1-HCP, (*S*)-(-)-limonene and TCA may possibly be ascribed to the reduced activities of ethylene biosynthesis enzymes and/or by the blocking of ethylene receptor sites irreversibly.

Like climacteric ethylene, rate of climacteric respiration was suppressed, and respiratory climacteric peak was delayed in both apple cultivars by all fumigation treatments with highly pronounced influence of 1-MCP (Table 4.3 and 4.4). (*S*)-(-)-limonene and TCA resulted in delayed respiratory peaks following 1-MCP in comparison to 1-HCP and control. Highly suppressed respiration rate by fumigation of ethylene antagonists can be related to the reduced ethylene production by these fumigation treatments (Ullah *et al.*, 2016). Since, a suppressed rate of respiration leads to a decline in ATP production, which is required for the conversion of ACC to ethylene (de Wild *et al.*, 1999), reduced ethylene production in apple cultivars in the current study caused by ethylene antagonists can be the result of reduced respiration rate.

Cold storage periods (28, 75 and 120 days) also affected the rate of climacteric ethylene production, respiration rate and their corresponding peaks. Cold storage was found to be effective with prior fumigation treatments of ethylene antagonists to reduce the climacteric rise of ethylene and respiration. However, extension of cold storage periods increased the climacteric ethylene production and respiration rate which increased even further during shelf storage of fruit. It is because with every 10 °C increase in temperature, metabolic processes in fruit increase 2 - 3 times (Saltveit, 2016).

Fumigation treatments were effective in maintaining fruit firmness following cold storage periods as compared to the control in both apple cultivars (Table 4.5). Higher fruit firmness was recorded in 1-MCP fumigated fruit followed by TCA, (*S*)-(-)-limonene and 1-HCP as compared to the control. Possibly, inhibition of fruit firmness loss with ethylene antagonists after cold storage could be due to suppressed rate of ethylene production as ethylene must be present continuously at required levels to initiate fruit softening (Watkins *et al.*, 2000). Retention of apple fruit firmness by ethylene antagonists has been reported earlier in several cultivars (Saftner *et al.*, 2003; Oraguzie *et al.*, 2007). TCA probably helped to retain fruit firmness and overall fruit quality by reducing the electrolyte leakage which normally happens after long term fruit storage and is an indication of senescence (Xing *et al.*, 2011).

Both ‘Fuji’ and ‘Cripps Pink’ had significantly higher TA in 1-MCP fumigated fruit which was followed by TCA and (*S*)-(-)-limonene fumigation treatments (Table 4.6 and 4.7). Considerably higher TA in 1-MCP, TCA and (*S*)-(-)-limonene fumigated fruit can be ascribed to reduced respiration rate by these ethylene antagonists and vice versa as there is a reported connection between TA and respiration rate of fruits. According to Lurie and Klein (1990) organic acids of fruits are responsible for their TA and their utilization as a substrate during respiration cause decline in TA. SSC of ‘Fuji’ was significantly higher in 1-MCP fumigated fruit while in ‘Cripps Pink’, significantly higher SSC was recorded in TCA and (*S*)-(-)-limonene fumigation treatments (Table 4.6 and 4.7). SSC percentage of 1-HCP fumigated ‘Cripps Pink’ fruit was at par with control fruit suggesting that ethylene action is not essentially related with accumulation of soluble solids as both ethylene-dependent and independent ripening processes exist in climacteric fruits (Lelievre *et al.*, 1997). An

increase in SSC in fruits is linked to the ripening process exhibiting the conversion of starch into the soluble sugars (Razzaq *et al.*, 2016).

Concentration of individual and total sugars in ‘Fuji’ did not vary much between 75 and 120 days cold stored fruit which is consistent with some previous reports (Suni *et al.*, 2000; Mikulic *et al.*, 2009a). A considerable increase in fructose content of ‘Cripps Pink’ was observed after long-term cold storage of 120 days which is supported by the earlier study of Veberic *et al.* (2010) on ‘Jonagold’ and ‘Golden Delicious’ apples. Concentration of individual and total sugars in ‘Fuji’ apple was significantly less with (S)-(-)-limonene fumigation treatment which can possibly be due to the delayed fruit ripening which ultimately reduced/delayed the conversion of starch into sugars (Razzaq *et al.*, 2016). Moreover, in both ‘Fuji’ and ‘Cripps Pink’ very minor changes in the levels of individual and total organic acids were observed with control and ethylene antagonist fumigation after 75 and 120 days cold storage (Table 4.8 – 4.11). It seems that reduced respiration rate with fumigation of ethylene antagonists resulted in maintaining the organic acid levels. An evasive impact of fumigation treatments on sugars and organic acids content of ‘Fuji’ and ‘Cripps Pink’ suggests that suppression of ethylene does not affect accumulation of these compounds (Pech *et al.*, 2012).

Ascorbic acid content was reduced in ‘Fuji’ and ‘Cripps Pink’ after 75 and 120 days CS to varying degrees with different ethylene antagonists except 1-MCP fumigation treatment which resulted in higher concentration of ascorbic acid followed by (S)-(-)-limonene fumigation. 1-MCP, 1-HCP and (S)-(-)-limonene also maintained relatively higher levels of total phenolics in comparison to TCA and control. Both ascorbic acid and total phenolics are known as the indicator of antioxidant capacity (Miller *et al.*, 1995). 1-MCP and TCA fumigation treatments resulted in comparatively higher antioxidant levels in ‘Fuji’ and ‘Cripps Pink’ cultivars (Table 4.12 and 4.13). Cinnamaldehyde has been reported to increase the activities of antioxidant enzymes i.e. peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) and reduced the oxidative stress in ‘Rose’. It also increased the concentration of soluble proteins improving flower quality (Jing *et al.*, 2011) which is probably related to its positive impact on total antioxidant activity and fruit quality in the present study.

In conclusion, different cold storage periods and ethylene antagonists significantly impacted the climacteric ethylene, respiration, onset of climacteric peaks and fruit quality parameters of ‘Fuji’ and ‘Cripps Pink’ apple. 1-MCP fumigation was more effective in reducing climacteric ethylene production, respiration rate, delayed climacteric peaks, inhibited the loss of fruit firmness and other tested quality parameters followed by 1-HCP, (*S*)-(-)-limonene and TCA.

### **7.3. Effect of Ethylene Antagonists on CA Storage of Apple cv. ‘Fuji’ and ‘Cripps Pink’**

In two different experiments, the effects of 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA fumigation on rate of climacteric ethylene production, respiration and quality parameters including SSC, TA, SSC/TA ratio, firmness, fruit peel colour, concentration of ascorbic acid, total phenolics, antioxidant capacity, total and individual sugars and organic acids in CA (2 % O<sub>2</sub> and 1 % CO<sub>2</sub> at 1 °C and 95 % R.H.) stored (6 and 8 months) ‘Fuji’ and ‘Cripps Pink’ apple fruit were investigated.

Among various fumigation treatments, 1-MCP and 1-HCP were found to be highly effective in reducing the production of climacteric ethylene and to delay the ethylene climacteric peaks in ‘Cripps Pink’ and ‘Fuji’ after 6 and 8 months of CA storage (Table 5.1 and 5.2). Possibly, application of 1-MCP may have reduced the rate of ethylene production by suppressing the expression of MaACS1, MaERS2, MaERS3, MaEIL1, MaEIL3 and MaEIL4 genes which are associated with ethylene signalling cascade (Yan *et al.*, 2011). 1-MCP fumigation has also been reported to reduce the climacteric ethylene in plum, apple and banana fruits (Abdi *et al.*, 1998; Janisiewicz *et al.* 2003; Yan *et al.*, 2011). As 1-HCP is a structural analogue of 1-MCP, its efficacy could be due to its structural similarity to 1-MCP or due to an extended chain length as stated by Sisler *et al.* (2003). 1-HCP has been proved effective to reduce the ethylene production in climacteric fruits of banana, avocado and tomato (Sisler *et al.*, 2003; Apelbaum *et al.*, 2008; Khan *et al.*, 2016).

All ethylene antagonists resulted in the suppressed respiratory climacteric in comparison to control after CA storage of both apple cultivars (Table 5.3 and 5.4). However, the effect of 1-MCP was more effective in reducing and delaying the respiration peaks as compared to all other ethylene antagonists and control. Previously,

apple, plum and papaya fruits treated with 1-MCP also exhibited reduced respiration rate (Rupasinghe *et al.*, 2000; Bai *et al.*, 2005; Dal Cin *et al.*, 2006; Martinez-Romero *et al.*, 2003; Fabi *et al.*, 2007). Reduction in the respiration rate with all the tested ethylene antagonists can be ascribed to the blockage of ethylene receptors and reduced ethylene production by these fumigation treatments. Higher concentration of CO<sub>2</sub> and low temperature during CA storage can also be responsible for lowering the respiration rate in ‘Fuji’ and ‘Cripps Pink’ apple as described by Ho *et al.* (2010).

Among fruit colour parameters, a\* was significantly improved with 1-HCP and (S)-(-)-limonene, while lightness (L\*) and yellowness (b\*) were reduced with fumigation of 1-MCP, 1-HCP and (S)-(-)-limonene (Table 5.5 and 5.6). Effect of ethylene antagonists in improving the colour is unclear as red colour in ‘Fuji’ and ‘Cripps Pink’ is due to anthocyanins and peaks of anthocyanins are associated with maturation phase of apple fruit when the concentration of ethylene begins to rise (Lister *et al.*, 1994; Awad *et al.*, 2001). All tested ethylene antagonists greatly inhibited the fruit softening in ‘Fuji’ and ‘Cripps Pink’, however, 1-MCP fumigated fruit exhibited higher fruit firmness as compared to all other fumigation treatments and control (Table 5.7 and 5.8). Positive effect of 1-MCP, 1-HCP, (S)-(-)-limonene and TCA to reduce the loss of firmness after CA storage in comparison to control can be referred to their varying levels of ethylene inhibitory action causing reduction in the amount of pectin degrading enzymes (PME) (Lohani *et al.*, 2004). Loss of firmness was generally slow during CA storage but increased sharply at shelf conditions probably due to variations in temperature and oxygen levels between storage and simulated shelf conditions (Gwanpua *et al.*, 2012).

1-MCP fumigated ‘Cripps Pink’ and ‘Fuji’ fruit showed relatively higher TA and SSC as compared to all other fumigation treatments and control; but 1-HCP, (S)-(-)-limonene and TCA were also effective in maintaining TA and SSC of apple fruit. SSC/TA ratio was maximum in control fruit of both ‘Fuji’ and ‘Cripps Pink’ after each CA storage period (Table 5.7 and 5.8), which represents the perception of fruit sweetness by consumers (Contessa and Botta, 2016). Biochemical components such as ascorbic acid, total phenolics, antioxidants, sugars and organic acids varied greatly between ‘Fuji’ and ‘Cripps Pink’ when assessed on 10<sup>th</sup> day of simulated shelf conditions. According to Contessa and Botta (2016), nutritional profile of fruit is

common for all apple genotypes, but different cultivars show considerable variations in nutritional components. All fumigation treatments with ethylene antagonists aided to maintain relatively higher concentration of ascorbic acid, total phenolics and total antioxidants in both apple cultivars than control. Effect of CA storage periods was not significant for ascorbic acid and total phenolics in ‘Cripps Pink’. However, generally low ascorbic acid concentration in ‘Cripps Pink’ after 8 months CA storage than 6 months stored fruit is consistent with the earlier report of Lee and Kader (2000) that ascorbic acid normally declines in fruit with prolonged storage and exposure to excessive CO<sub>2</sub> which promotes oxidation of ascorbic acid.

Higher concentration of glucose, fructose and total sugars in ‘Cripps Pink’ was noted with 1-MCP fumigation while in ‘Fuji’, concentrations of these sugars were predominantly high with (*S*)-(-)-limonene fumigation. Conversely, there was no discrete effect of ethylene antagonists on sucrose levels in ‘Cripps Pink’ and ‘Fuji’ fruit (Table 5.9 and 5.10). Fructose level reduced after 8 months CA storage in comparison to 6 months CA stored fruit of ‘Cripps Pink’ which can lead to a reduction in fruit sweetness as fructose is the sweetest sugar as compared to sucrose and glucose (Contessa and Botta, 2016). Among organic acids, malic acid concentration was high in both apple cultivars regardless of CA storage period as malic acid comprises 90 % of apple’s acid content while others are present in trace amounts (Ackermann *et al.*, 1992). Citric acid was almost parallel in concentration to malic acid in ‘Cripps Pink’ after 6 and 8 months storage in CA; however, it was not detected in ‘Fuji’ fruit after 6 months CA storage. Concentration of malic, succinic, fumaric and total organic acids was found non-significant in ‘Fuji’ after 6 months of CA storage (Table 5.11 and 5.12). The variation in responses to tested ethylene antagonists in this study could be referred to the differential effect of these compounds on expression of ripening related genes and turnover of ethylene receptors after fumigation (Ziliotto *et al.*, 2008).

In conclusion, although 1-MCP has most effectively reduced climacteric ethylene production, respiration rate and maintained fruit quality of CA stored ‘Fuji’ and ‘Cripps Pink’ apple, findings of the current study suggest that 1-HCP, (*S*)-(-)-limonene and TCA can also be used in commercial apple industry to avoid the shortcomings of 1-MCP in various CA stored apple cultivars. Moreover, the

mechanism for the effect of ethylene antagonists on sugar and organic acid's metabolism needs to be investigated in future studies.

#### **7.4. Effect of Ethylene Antagonists on Cold Storage Life and Fruit Quality of Pear cv. 'Packham's Triumph' and 'Beurre Bosc'**

These experiments were conducted to evaluate the most potent ethylene antagonist and storage regimes to extend the cold storage life of pear cultivars with better quality fruit. For this purpose, pear fruit of cv. 'Packham's Triumph' and 'Beurré Bosc' were fumigated with 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA in two independent experiments. After fumigation of pear fruit with ethylene antagonists, both treated and untreated control fruit were stored in cold rooms at 0 – 1 °C and > 85 ± 5 % R.H. for the period of 4 and 6 months. After cold storage periods, pear fruit were moved to simulated shelf conditions (21 ± 1 °C) and ethylene production and respiration rate were recorded daily until post-climacteric stage. Quality parameters viz. firmness, colour, TA, SSC, SSC/TA ratio, individual and total sugars, organic acids, ascorbic acid and total antioxidants were assayed on 10<sup>th</sup> day of simulated shelf conditions.

In both pear cultivars, 1-MCP fumigation was found to be highly effective in reducing the rate of climacteric ethylene production and delaying the ethylene climacteric peak as compared to other ethylene antagonists and control (Table 6.1 and 6.2). Following 1-MCP, 1-HCP also suppressed ethylene production at climacteric peak in 'Packham's Triumph', while in 'Beurré Bosc' (*S*)-(-)-limonene fumigation led to the reduced rate of climacteric ethylene production. Moreover, (*S*)-(-)-limonene and TCA fumigation effectively delayed the onset of climacteric peak of ethylene in 'Packham's Triumph' and 'Beurré Bosc' pear. Onset of respiratory climacteric peak and rate of respiration at climacteric was significantly delayed and suppressed in both pear cultivars by 1-MCP fumigation as compared to all other ethylene antagonists and control. In addition, 1-HCP, (*S*)-(-)-limonene and TCA fumigation also effectively reduced the respiration rate and delayed the respiratory climacteric peaks in comparison to control (Table 6.3 and 6.4).

Comparatively reduced and delayed ethylene climacteric with ethylene antagonists and ultimately reduced respiration rate in comparison to control could be

due to the stabilization of ethylene receptor proteins by ethylene antagonists which ethylene cannot degrade, while antagonists are bind to the receptor sites. Consequently, ripening and expression of related genes seems to be delayed (Kevany *et al.*, 2007 and 2008). Specifically, 1-MCP has been reported to interfere with the mechanism of autocatalytic ethylene biosynthesis (Razzaq *et al.*, 2016). Reduced rate of climacteric ethylene production and respiration rates can also be ascribed to low temperature of cold storage in combination with ethylene antagonists as cooling temperatures between 1 and 8 °C lead to highly reduced enzymatic activities in stored commodities (Reque *et al.*, 2014). Rate of climacteric ethylene production reduced after 6 months cold storage in both pear cultivars in comparison to 4 months. It can be explained by the fact that in pears ethylene peaks approach earlier with higher magnitudes and with an extension of cold storage periods, rate of climacteric ethylene declines (Wang and Sugar, 2013).

Fruit firmness of tested pear cultivars was effectively retained with 1-MCP fumigation treatments followed by TCA in ‘Packham’s Triumph’ and with 1-HCP in ‘Beurre Bosc’ pear (Table 6.5). Softening of fruit results from an increase in the activity of enzymes which degrade the cell wall. Particularly, in pear fruit, the activity of PG and endo-1,4- $\beta$ -glucanase enzymes increase sharply during ripening along with solubilisation of pectin and hemicellulose in cell wall, ultimately resulting in soft fruit. Ethylene plays an important role in these processes of fruit softening (Hiwasa *et al.*, 2003). In the current study, cold storage in combination with ethylene antagonists delayed fruit softening in pear. Similar results have been reported earlier by Itai and Tanahashi (2008) in Japanese pear. An inhibition of fruit firmness by 1-MCP in the present study is also supported by previous work on nectarine, peach and plum whereby 1-MCP application reduced the activities of fruit softening enzymes (Khan and Singh, 2007; Ortiz *et al.*, 2011; Ullah *et al.*, 2016). 1-HCP fumigation in the current study may possibly has also suppressed the activities of fruit softening enzymes in pear thus inhibiting the sharp decline of fruit firmness.

In both pear cultivars, higher fruit TA was recorded with all fumigation treatments in comparison to control. However, with an extended cold storage period from 4 to 6 months, reduction in TA was found. As malic acid is used in tri-carboxylic acid cycle during the process of respiration, TA of fruit declines after prolonged

storage. Hence, increased TA with ethylene antagonists is probably due to reduced respiration rate with fumigation treatments (Chen *et al.*, 1982; Wang and Sugar, 2013). No difference among fumigation treatments and control was found for SSC but maximum SSC/TA ratio was observed in control fruit due to low values of TA. Extension of cold storage period resulted in an increased SSC and SSC/TA ratio of pear fruit (Table 6.6 and 6.7).

Individual and total sugars of ‘Packham’s Triumph’ and ‘Beurré Bosc’ pears varied between 4 and 6 months cold stored fruit, among fumigation treatments and control fruit. However, the effect of ethylene antagonists on concentration of sugars in pear cultivars was not discrete (Table 6.8 and 6.9). Fructose concentration of ‘Packham’s Triumph’ and ‘Beurré Bosc’ was generally high compared to sucrose and glucose. However, differences in fructose level between two pear cultivars after 4 and 6 months CS can be ascribed to the varying activity of sugar degrading and synthesizing enzymes (Yamada *et al.*, 2006). Effect of ethylene antagonists and cold storage periods was non-significant in ‘Packham’s Triumph’ for individual and total organic acids. In ‘Beurré Bosc’ pear, (*S*)-(-)-limonene and TCA fumigation resulted in slightly higher organic acid concentration than all other ethylene antagonists and control (Table 6.10 and 6.11). Antioxidant activity of ‘Packham’s Triumph’ and ‘Beurré Bosc’ pear was significantly high with (*S*)-(-)-limonene and TCA fumigation as compared to 1-MCP, 1-HCP and control. On the other hand, all fumigation treatments resulted in comparatively higher ascorbic acid concentration in pear cultivars than control (Table 6.12 and 6.13). During the process of fruit ripening and senescence, ascorbic acid content usually decreases, and this phenomenon is accelerated by the presence of oxygen. Ethylene antagonists slow down the ripening of fruits, reduce the respiration rate and may also reduce the diffusion of oxygen which ultimately inhibits the quick loss of ascorbic acid (Xing *et al.*, 2011).

In conclusion, positive impact of 1-MCP on pear fruit quality in present study is consistent with multiple previous studies which show that 1-MCP impedes ripening process in many pear cultivars (Lelièvre *et al.*, 1997; Hiwasa *et al.*, 2003; Argenta *et al.*, 2003; Trincherro *et al.*, 2004; Calvo and Sozzi, 2004). However, results of the present study also indicate that 1-HCP, (*S*)-(-)-limonene and TCA can also retard the ripening process in pear fruit by suppressing and delaying the ethylene and respiration

climacterics and inhibiting the loss of fruit firmness, colour and biochemical components, depending upon cold storage periods.

### 7.5. Conclusions

Ethylene antagonists control the ethylene action and fruit ripening processes at molecular and physiological level. However, highly variable responses of apple and pear cultivars towards a specific ethylene antagonist can be ascribed to the tissue specific variations in ethylene receptor attributes and time taken to synthesize new receptors. Results of the current study are briefly concluded below;

- Among all tested ethylene antagonists, 1-MCP was found most effective to inhibit ethylene action and to prolong storage life of treated fruit with high quality retention. Nonetheless, 1-HCP, (*S*)-(-)-limonene and TCA also played an effective role to maintain the fruit quality of apple and pear fruits while extending their storage life in comparison to control. However, efficacy of all ethylene antagonists was highly dependent on storage period, conditions and cultivars.
- After various cold storage periods of ‘Fuji’ and ‘Cripps Pink’, ethylene antagonists suppressed and delayed the ethylene and respiratory climacteric and helped to maintain the fruit quality parameters. 1-MCP fumigation led to the maximum suppression in climacteric ethylene production followed by (*S*)-(-)-limonene and TCA after 28 days CS of ‘Fuji’ fruit with delayed climacteric peak in both apple cultivars. Maximum delay in respiratory climacteric peak was found with (*S*)-(-)-limonene fumigation in ‘Cripps Pink’ apple as compared to the control. Ethylene antagonists did not have any obvious effect on TA, SSC and SSC/TA ratio but fruit firmness was retained to varying levels by all fumigation treatments depending upon cold storage durations. Ethylene antagonists helped to maintain the biochemical attributes of ‘Fuji’ and ‘Cripps Pink’ after cold storage i.e. ascorbic acid, total phenolics, total antioxidants and sugars as compared to the control. However, concentration of individual and total organic acids was not affected by ethylene antagonists significantly.

- After CA storage of 6 and 8 months, rate of climacteric ethylene production and respiration rate were suppressed and delayed in ‘Fuji’ and ‘Cripps Pink’ apple by fumigation with ethylene antagonists as compared to the untreated fruit. The losses of fruit peel colour, firmness, TA, SSC and their mutual ratio were also inhibited by ethylene antagonists in comparison to the control fruit. Fumigation treatments of 1-MCP, 1-HCP, (*S*)-(-)-limonene and TCA also exhibited maintained sugar levels, ascorbic acid, total phenolics and antioxidant capacity in both apple cultivars. On the other hand, organic acid concentrations were not significantly affected by fumigation of ethylene antagonists.
- After 4 and 6 months cold storage of ‘Packham’s Triumph’ and ‘Beurré Bosc’ pear cultivars, ethylene antagonists maintained the fruit quality better than control. Rate of climacteric ethylene production and respiration rate was highly suppressed while, onset of ethylene and respiratory climacteric peaks was delayed primarily with 1-MCP fumigation treatment as compared to other ethylene antagonists and control. Effect of 1-HCP, (*S*)-(-)-limonene and TCA was also positive as these fumigation treatments effectively maintained the firmness level, ascorbic acid concentration and total antioxidants as compared to the control in both pear cultivars. Concentrations of individual and total organic acids were not highly affected by fumigation treatments and cold storage periods.
- In brief, fruit quality and storage life of apple (cv. ‘Fuji’ and ‘Cripps Pink’) and pear (cv. ‘Packham’s Triumph’ and ‘Beurré Bosc’) was greatly affected when treated with ethylene antagonists depending upon storage type and storage duration. 1-MCP was found more effective to suppress the climacteric rise of ethylene production and respiration rates as well as to maintain the fruit quality parameters; however, 1-HCP, (*S*)-(-)-limonene and TCA were also effective to varying extent to prolong the storability and to keep the quality of apple and pear cultivars in the present study.

### 7.6. Recommendations for Future Research Work

Inappropriate storage conditions and ineffective postharvest treatments can greatly reduce the fruit quality after longer storage period (Wang and Sugar, 2013). Hence, optimization of storage regimes and the most potent ethylene antagonist is needed for commercial cultivars of apple and pear. There are a few recommendations for future research work to extend storage life and quality of pome fruits.

- Future research efforts should be directed to evaluate the cultivar specific responses of different horticultural crops towards new ethylene antagonists.
- Molecular level studies are needed to identify the differential selectivity of ethylene receptor sites for new compounds.
- Effect of 1-HCP, (*S*)-(-)-limonene and TCA on the expression of ripening related genes and activities of enzymes should be evaluated in future studies.
- Effect of these antagonistic compounds at various maturity stages of fruit in relation to different concentration levels and exposure times needs to be assessed.

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## Appendices

### Appendix # 1

10/3/2018 Mail - mehwish.yaseen@postgrad.curtin.edu.au

Reply all |

Alison Barber <abarber@apal.org.au>  
Tue 17/07, 1:38 PM  
Mehwish Yaseen

Flag for follow up. Start by Wednesday, 18 July 2018. Due by Wednesday, 18 July 2018.

| Action Items

Mehwish, we are happy for you to use images of apples and pears reproduced from the APAL site for the purpose of your thesis.

Please credit Apple and Pear Australia as the source of the images.

Note - it is Packham's Triumph, not Peckham's Triumph.

*Alison*



**Alison Barber**  
Manager, Communications & Media, Apple and Pear Australia Ltd.  
Suite G.01, 128 Jolimont Rd, East Melbourne, VIC 3002  
03 8652 5433 | 0424 004 070 | [abarber@apal.org.au](mailto:abarber@apal.org.au)

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Appendix # 2

10/3/2018

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Mehwish Yaseen

Reply all |

Tue 10/07, 12:46 PM

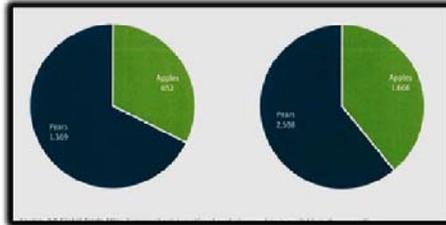
Tim.Foulds@euromonitor.com

Hi

I am a PhD student of postharvest horticulture from Curtin university. I have done my research on apple and pear fruits and now I am writing my thesis. I have included an image from apple and pear Q1 report of 2017.

I need permission for this reprinting as otherwise i would face problem from university. I would be thankful if you can provide me any permission document. I am pasting the image here for your reference. I edited it myself.

Thanking in anticipation. Regards,  
Mehwish



10/3/2018

Mail - mehwish.yaseen@postgrad.curtin.edu.au

Permission to reprint image from apple and pear report

Mehwish Yaseen

Wed 11/07, 10:42 AM

Dear Adam & Tim  
Thanks for your positive response.  
Surely i would cite the reference in my thesis.

Best Regards,  
Mehwish

AB

Adam Briggs <Adam.Briggs@horticulture.com.au>

Tue 10/07, 2:26 PM

Hi Tim,

All is well here. Nice to hear from you.

Mehwish, permission is granted to use the image. Please reference it accordingly in your research.

Regards,

Adam

**Adam Briggs** | Senior Analyst | Hort Innovation | Level 8, 1 Chifley Square, Sydney NSW 2000 | Ph: +612 8295 2335 | Mob: +61 409 578 937 | Fax: 02 8295 2399 | Email: adam.briggs@horticulture.com.au

TF

Tim Foulds <Tim.Foulds@Euromonitor.com>

Tue 10/07, 2:20 PM

Hi Adam,

I trust all is well.

Would you be able to confirm that Mehwish can use the said image?

Thanks,

Tim

Appendix # 3

10/3/2018

Mail - mehwish.yaseen@postgrad.curtin.edu.au

Angus Crawford <acrawford@apal.org.au>  
Tue 10/07, 12:22 PM

Hi Mehwish

You have permission to use the growing regions map from the APAL website.

All the best.

Regards  
Angus



**Angus Crawford**

Technical Manager, Apple and Pear Australia Ltd.  
Suite G.01, 128 Jolimont Rd, East Melbourne, VIC, 3002  
Direct line 03 8652 5436 or APAL 03 9329 3511

Mobile 0427 111 852

[acrawford@apal.org.au](mailto:acrawford@apal.org.au)

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Appendix # 4

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**Brian Lipinski** <blipinski@wri.org>  
 Fri 13/07, 7:43 PM

Hello Mehwish,

Thanks for reaching out – I’m glad you found the paper useful. Yes, you can reprint the image, just make sure to cite the paper as the original source. We don’t have any sort of “official” permission document so you can consider this email your permission. Best of luck finishing the PhD!

Best,  
 Brian

---

**Mehwish Yaseen** |  
 Wed 11/07, 1:20 PM

Dear Lipinski,  
 Hope you will be doing great.  
 I am a PhD student of postharvest horticulture from Curtin university. I have done my research on apple and pear fruits and now I am writing my thesis. I have included an image from your working paper titled 'Reducing Food Loss and Waste' , 2013.  
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 Thanking in anticipation. Regards,  
 Mehwish

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Meat	10%
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Perth, WA  
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28 September 2018

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