School of Molecular and Life Sciences

Postharvest Interventions Involving Novel Ethylene Antagonists, Ozone and AiroFresh® in Regulating Ethylene and Maintaining Quality of Apple and Pear Fruits

Vijay Yadav Tokala

This thesis is presented for the Degree of

Doctor of Philosophy

of

Curtin University

August 2019

Declaration

To the best of my knowledge and belief, this thesis does not contain any previously published material by any other person, except those where due acknowledgements were made.

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

Signature: 

Date: **19th August 2019**

Dedication

Dedicated to Mother Nature, who is a source of wisdom, inspiration, strength, knowledge and everything necessary for my life.

Mother Nature...

You are the eternal thing...

the one who creates, the one who destroys,

the one who forms, the one who reforms,

the one who hurts, the one who soothes,

the one who teach, the one who test,

the one who forgives, the one who punishes,

I believe in you and I love you

There is neither a deity nor a messenger behalf of you, as you blessed every creature, in the same way, to know you and to understand you

I know that you understand every word of mine, after all you are the one who created language for every living on the earth.

Ohhh Mother Nature!! Bless me with your peace and serenity.

- Vijay Yadav

Acknowledgements

यः पठति लिखति पश्यति
परिपृच्छति पंडितान् उपाश्रयति।
तस्य दिवाकरकिरणैः नलिनी
दलं इव विस्तारिता बुद्धिः॥

Yah pathati, likhati, pashyati
paripruchchhati panditaan
upaashrayati.
Tasya divaakarakiranaih nalinee
dalan iva vistaarita buddhih.

The *shloka* (verse) from *Sanskrit Subhāşitas* states that "One who reads, writes, sees, inquires, lives in the company of learned, his intellect expands similar to the lotus petals which expands with the rays of the sun." During my PhD research, I was fortunate enough to have the support of several intellectuals and a great chance to expand my knowledge. Out of all, I would like to put in first position, with immense gratitude, my esteemed supervisor Professor Zora Singh for imparting his mammoth experience, extensive knowledge and skills in me and for his caring behavior. He has provided me the guidance till completion of my research and thesis writing. Professor Singh made continuous efforts to accommodate a supportive environment in spite of his multifarious responsibilities and his research supervision was very infectious..

It gives me great pleasure in acknowledging the support and help of Dr Alan Payne, for his generous gestures and critical suggestions throughout the course of investigation and thesis writing. I express my cordial thanks to Dr Ranil Coorey, Assoc. Prof. Monique Gagnon, Prof. Ravi Fotedar and Prof. Satvinder Dhaliwal for providing their valuable suggestions and moral support during critical times of degree. I extend sincere thanks to Ms Susan Petersen for all the technical support provided during the experiments.

I wish to express my gratitude and deepest sense of affection to my mentor Dr Lisa Kitinoja, Dr Basharat Ali Saleem, Prof. Maliram Choudhary and Dr S.S. Vijaya Padma for their constant love, critical suggestions, intellectual insights and academic foresight from time to time to help to reach my goal in time.

It would have been a very tough job for me to complete my PhD journey without the continuous support of my best friend and colleague Ms Poe Nandar Kyaw. I am beholden to my colleagues Dr Sabah, Dr Mekala, Dr Muneer, Dr Mehwish and Ms Rahil; my close friends Sameer, Chetana, Prathibha and Pandey for their regular moral support, motivation and inspiration.

I would like to express the deepest sense of reverence to my parents Prof. T. Sambaiah and Ms T. Vijaya Lakshmi, who gave me my name, my life, and everything else in between. I will always be indebted for their endless love and efforts they have put into giving me the life I have now. I also express my deep affection to my brother Ajay Yadav, sister Niveditha, brother-in-law Dr Nagaraju Yadav and all other well-wishers whose incessant love, affection and encouragement brought the present task to completion.

I am thankful to Australian Government and Curtin University for awarding me the Australian Government Research Training Program Scholarship (formerly known as International Postgraduate Research Scholarship). The Western Australian fruit growers, distributors and storage - 'The Newton Brothers Orchards, Manjimup'; 'Eastwind Farms, Balingup'; 'Casuarina Valley Orchards, Beedelup'; 'Bluemoon Packing, Kirup'; 'Della Pollina Orchards, Pickering Brook' and 'CA Storage, Casuarina Orchards, Carmel' are gratefully acknowledged for providing the experiment fruit and access to the storage facilities.

The following works have been published/presented:

Book chapter: **Vijay Yadav Tokala**, Zora Singh and Alan D. Payne. 2018. Postharvest uses of ozone application in fresh horticultural produce. In: Postharvest Biology and Nanotechnology (Eds. G. Paliyath, J. Subramanian, L-T Lim, K.S. Subramanian, A. Handa and A. Matoo). John Wiley and Sons, Inc. pp. 129-170.

Conference talk: **Vijay Yadav Tokala**, Zora Singh, Alan D. Payne and Poe Nandar Kyaw. 2019. New ethylene antagonists regulate ethylene action and maintain fruit quality in long term-controlled atmosphere stored Gold Rush pear presented at "2019 ASHS Annual Conference | July 21 - 25, 2019 | Las Vegas, NV, USA":

Sincerely

Vijay Yadav Tokala

General abstract

The United Nations Food and Agriculture Organisation (UNFAO) in 2011 has estimated that nearly one-third of all the food and about one-half of all the fruit and vegetables produced for human consumption is either lost or wasted at different stages of the supply chain. The rate of postharvest physiological activities associated with the ripening process is very high in the horticultural crops. This property makes the fruit and vegetables highly susceptible to rapid quality deterioration and also responsible for up to 50% of all the postharvest losses (Blanke, 2014). The phytohormone ethylene plays a key role in the fruit ripening process and ultimately in senescence and deterioration. Appropriate postharvest techniques to delay the fruit ripening and to regulate the ethylene during storage, significantly reduce the postharvest losses, extend the storage life as well as maintain consumer-preference fruit quality. The efficacy of novel ethylene antagonists namely 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) as well as widely used 1-methylcyclopropene (1-MCP) in retarding the rates of respiration and ethylene production and in maintaining the optimum fruit quality in Granny Smith apple, Cripps Pink apple and Gold Rush fruit stored in cold or controlled atmosphere (CA) storage with or without ozone or AiroFresh® was investigated. The experiments were designed using storage environments used by the fruit growers, for the results to be more relevant and applicable to them.

The Cripps Pink apple fruit were fumigated with 1 µM BC, 1 µM NC, or 18 µM (1 µL L^{-1}) 1-MCP for 18 h or dipped in 20 L aqueous solution of 5 % ethanol only, 2 µM BC in 5 % ethanol or 2 µM NC in 5 % ethanol for 5 min (at 20±2 °C) and stored in cold storage for 100 and 150 days. The fumigation and dip formulations of the ethylene antagonists retarded the rates of ethylene production, reduced physiological loss of weight (PLW) as well as maintained higher firmness, total phenols and titratable acidity in the fruit when compared to control and ethanol treatments following 100 and 150 days of storage. The BC and NC fumigation treatments performed better than respective dip treatments in retarding ethylene and in maintaining the fruit quality.

The Granny Smith apple fruit fumigated with 1-MCP and the Cripps Pink apple fruit fumigated with BC and NC exhibited reduced rates of ethylene and respiration climacteric peaks following 90 and 120 days of cold storage. The Cripps Pink and

Granny Smith apple fruit fumigated with BC, NC and 1-MCP exhibited reduced PLW and higher fruit firmness as well as higher levels of total phenols and total antioxidant capacity when compared to control fruit. A rise in rates of ethylene and respiration climacteric peaks was recorded in the Granny Smith and Cripps Pink apple fruit when stored in ozonated cold storage. The ozone application in cold storage reduced PLW and increased individual sugar levels in both the apple cultivars studied. No significant interaction effect between the ethylene antagonist fumigation and ozonated cold storage was observed on the rates of ethylene production and respiration as well as on other fruit quality parameters in both the apple cultivars.

The Cripps Pink apple fruit fumigated with BC, NC and 1-MCP exhibited reduced rates and delayed onset of the respiration and ethylene climacteric peaks as well as maintained higher fruit firmness and total phenol levels when compared to the control fruit during the CA storage. The Cripps Pink fruit stored in the CA storage with AiroFresh® showed reduced rates of respiration and ethylene climacteric peaks, lower PLW as well as higher fruit firmness and total antioxidant capacity levels when compared to the fruit stored in CA storage without AiroFresh® following 90 days of storage. Contrarily, following 120 days of storage the Cripps Pink fruit stored in the CA storage with AiroFresh® showed higher rates of respiration and ethylene climacteric peaks than the fruit stored in CA storage without AiroFresh®. No significant interaction effect between ethylene antagonist treatments and type of CA storage on the respiration and ethylene rates as well as other fruit quality parameters was observed.

The Granny Smith apple fruit fumigated with BC, NC and 1-MCP exhibited retarded rates and delayed onset of respiratory and ethylene climacteric peaks, retained higher fruit firmness and showed lower PLW when compared to the control fruit following 90 and 120 days of CA storage. The ethylene antagonist treatments in Granny Smith apple maintained higher levels of SSC, individual sugars and acids, total phenols, ascorbic acid as well as total antioxidant capacity in comparison with the control fruit. Similarly, the fumigation treatment with BC, NC and 1-MCP significantly reduced the rates of ethylene and respiratory climacteric peaks as well as maintained higher fruit firmness and lower PLW when compared to control in the Gold Rush pear fruit stored in CA storage for 150 and 200 days. The fumigation with 1-MCP retarded ethylene

and respiration rates more effectively than BC and NC treatments in CA stored Granny Smith apple and Gold Rush pear fruit.

In conclusion, the novel ethylene antagonists BC and NC possess potential to be used as an alternative to 1-MCP in Cripps Pink apple, Granny Smith apple and Gold Rush pear fruit during cold or CA storage with or without ozone or AiroFresh®.

Table of Contents

Declaration **i**

Dedication **ii**

Acknowledgements **iii**

General abstract **v**

Table of contents **viii**

List of figures **xvii**

List of tables **xxi**

List of symbols and abbreviations **xxvii**

Chapter 1 General introduction **1**

General objective 6

Specific objectives 6

Chapter 2 General review of literature **7**

2.1 Introduction 7

2.2 Economic importance of apples and pears 8

2.2.1 World statistics 8

2.2.2 Australian statistics 10

2.2.3 Major commercial cultivars of apple and pear 12

2.3 Postharvest losses 14

2.4 Fruit ripening 16

2.4.1 Respiration 16

2.4.2 Fruit colour 17

2.4.3 Flavour development 18

2.4.4 Fruit softening 19

2.5 Ethylene 20

2.5.1 History 20

2.5.2 Biochemistry of ethylene biosynthetic pathway 21

2.5.3 Physiological effects of ethylene 22

2.5.4 Ethylene signal transduction 24

2.5.5 Ethylene agonists 25

2.5.6 Ethylene regulation 25

2.5.6.1 Inhibition of ethylene biosynthesis 26

2.5.6.2 Inhibition of ethylene action 27

a. Cycloalkenes 28
b. Terpenes 28
c. Cyclopropenes 28
2.6 1-Methylcylopropene (1-MCP) 29
2.6.1 Mode of action of 1-MCP 31
2.6.2 Effects of 1-MCP on postharvest physiology and quality of the fruit 31
2.7 Novel ethylene antagonists 33
2.7.1 Proposed mode of action 34
2.8 Storage environments 34
2.8.1 Cold storage 34
2.8.2 Controlled atmospheric (CA) storage 36
2.9 Ozone 37
2.10 AiroFresh® 41
Chapter 3 General materials and methods 42
3.1 Fruit material 42
3.2 Ethylene antagonists 44
3.3. Postharvest treatments with ethylene antagonists 45
3.3.1. Ethylene antagonist fumigation treatment 45
3.3.2. Ethylene antagonist dip treatment 45
3.4. Storage conditions 46
3.4.1. Controlled atmosphere (CA) storage 46
3.4.2 Cold storage 46
3.4.3. Ozone 47
3.4.4. AiroFresh® 49
3.5 Determination of fruit physiological parameters 49
3.5.1 Ethylene (C_2H_4) production rate 50
3.5.2 Respiration rate 52
3.6 Determination of fruit quality parameters 53
3.6.1 Physiological loss of weight (PLW) 53
3.6.2 Fruit firmness 53
3.6.3 Soluble solid concentration (SSC), titratable acidity (TA) and SSC: TA ratio 54

3.6.4 Fruit sampling 55
3.6.5 Individual sugars and organic acids 55
3.6.5.1 Preparation of sample 55
3.6.5.2 HPLC system 56
3.6.6 Total phenols 59
3.6.7 Ascorbic acid 60
3.6.8 Total antioxidant capacity 62
3.7 Statistical analysis of data 62
Chapter 4 Effect of the different formulations of novel ethylene antagonists on the ethylene production and fruit quality of cold-stored Cripps Pink apples **63**
Abstract 63
4.1 Introduction 63
4.2 Material and methods 65
4.2.1 Fruit and experiment conditions 65
4.2.2 Determination of fruit physiological parameters 66
4.2.2.1 Rate of ethylene production 66
4.2.2.2 Rate of respiration 66
4.2.3 Determination of fruit quality parameters 66
4.2.3.1 Physiological loss of weight (PLW) 66
4.2.3.2 Fruit firmness 67
4.2.3.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA 67
4.2.3.4 Individual sugars and organic acids 67
4.2.3.5 Total phenols 68
4.2.3.6 Ascorbic acid 68
4.2.3.7 Total antioxidant capacity 68
4.2.4 Statistical analysis 68
4.3 Results 69
4.3.1 Fruit physiological parameters 69
4.3.1.1 Ethylene (C_2H_4) production 69
4.3.1.2 Respiration (CO_2 production) 69
4.3.2 Fruit quality parameters 72

4.3.2.1 Physiological loss of weight (PLW) 72
4.3.2.2 Fruit firmness 72
4.3.2.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA 74
4.3.2.4 Individual sugars 76
4.3.2.5 Individual organic acids 76
4.3.2.6 Total phenols, ascorbic acid and total antioxidant capacity 80
4.4 Discussion 82
4.5 Conclusion 84
Chapter 5 Effects of novel ethylene antagonists and ozone in regulating ethylene production and fruit quality of cold-stored Cripps Pink and Granny Smith apples **86**
Abstract 86
5.1 Introduction 87
5.2 Material and methods 89
5.2.1 Fruit and experimental conditions 89
5.2.1.1 Experiment 1: Effects of novel ethylene antagonists and ozone on postharvest physiology and quality of cold-stored Cripps Pink apple fruit 89
5.2.1.2 Experiment 2: Effects of novel ethylene antagonists and ozone on postharvest physiology and quality of cold-stored Granny Smith apple fruit 90
5.2.2 Determination of fruit physiological parameters 90
5.2.2.1 Rate of ethylene production 90
5.2.2.2 Rate of respiration 91
5.2.3 Determination of fruit quality parameters 91
5.2.3.1 Physiological loss of weight (PLW) 91
5.2.3.2 Fruit firmness 91
5.2.3.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA 91
5.2.3.4 Individual sugars and organic acids 92
5.2.3.5 Total phenols 92

5.2.3.6 Ascorbic acid 92
5.2.3.7 Total antioxidant capacity 92
5.2.4 Statistical analysis 93
5.3 Results 93
5.3.1 Fruit physiological parameters 93
5.3.1.1 Ethylene (C_2H_4) production 93
5.3.1.2 Respiration (CO_2 production) 94
5.3.2 Fruit quality parameters 98
5.3.2.1 Physiological loss of weight (PLW) 98
5.3.2.2 Fruit firmness 98
5.3.2.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA 101
5.3.2.4 Individual sugars 104
5.3.2.5 Individual organic acids 105
5.3.2.6 Total phenols 110
5.3.2.7 Ascorbic acid 110
5.3.2.8 Total antioxidant capacity 111
5.4 Discussion 114
5.5 Conclusions 116
Chapter 6 Effects of novel ethylene antagonists and AiroFresh® in regulating ethylene production and fruit quality of Cripps Pink apples stored in controlled atmosphere storage **118**
Abstract 118
6.1 Introduction 119
6.2 Material and methods 121
6.2.1 Fruit and experimental conditions 121
6.2.2 Determination of fruit physiological parameters 122
6.2.2.1 Rate of ethylene production 122
6.2.2.2 Rate of respiration 122
6.2.3 Determination of fruit quality parameters 122
6.2.3.1 Physiological loss of weight (PLW) 122
6.2.3.2 Fruit firmness 123

6.2.3.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA 123
6.2.3.4 Individual sugars and organic acids 123
6.2.3.5 Total phenols 123
6.2.3.6 Ascorbic acid 124
6.2.3.7 Total antioxidant capacity 124
6.2.4 Statistical analysis 124
6.3 Results 124
6.3.1 Fruit physiological parameters 124
6.3.1.1 Ethylene (C_2H_4) production 124
6.3.1.2 Respiration (CO_2 production) 125
6.3.2 Fruit quality parameters 129
6.3.2.1 Physiological loss of weight (PLW) 129
6.3.2.2 Fruit firmness 129
6.3.2.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA 131
6.3.2.4 Individual sugars 133
6.3.2.5 Individual organic acids 135
6.3.2.6 Total phenols, ascorbic acid and total antioxidant capacity 135
6.4 Discussion 139
6.5 Conclusions 141
Chapter 7 Efficacy of postharvest fumigation with novel ethylene antagonists on ethylene production and fruit quality of Granny Smith and Cripps Pink apples stored in different controlled atmosphere storages **143**
Abstract 143
7.1 Introduction 144
7.2 Material and methods 146
7.2.1 Fruit and experimental conditions 146
7.2.1.1 Experiment 1: Effects of new ethylene antagonist treatments on ethylene production and postharvest 146

quality of Granny Smith apple fruit stored in controlled atmospheric storage

7.2.1.2 Experiment 2: Effects of fumigation with novel ethylene antagonists on the postharvest physiology and quality of Cripps Pink apple fruit stored in controlled atmospheric storage with AiroFresh® 147

7.2.2 Determination of fruit physiological parameters 148

7.2.2.1 Rate of ethylene production 148

7.2.2.2 Rate of respiration 148

7.2.3 Determination of fruit quality parameters 149

7.2.3.1 Physiological loss of weight (PLW) 149

7.2.3.2 Fruit firmness 149

7.2.3.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA 149

7.2.3.4 Individual sugars and organic acids 149

7.2.3.5 Total phenols 150

7.2.3.6 Ascorbic acid 150

7.2.3.7 Total antioxidant capacity 150

7.2.4 Statistical analysis 150

7.3 Results 150

7.3.1 Fruit physiological parameters 150

7.3.1.1 Ethylene (C_2H_4) production 150

7.3.1.2 Respiration (CO_2 production) 151

7.3.2 Fruit quality parameters 155

7.3.2.1 Physiological loss of weight (PLW) 155

7.3.2.2 Fruit firmness 155

7.3.2.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA 158

7.3.2.4 Individual sugars 160

7.3.2.5 Individual organic acids 164

7.3.2.6 Total phenols 166

7.3.2.7 Ascorbic acid 166

7.3.2.8 Total antioxidant capacity 166

7.4 Discussion 168
7.5 Conclusions 171
Chapter 8 Efficacy of postharvest fumigation of novel ethylene antagonists in regulating ethylene production and fruit quality of Gold Rush pears during controlled atmosphere storage **172**
Abstract 172
8.1 Introduction 173
8.2 Material and methods 174
8.2.1 Fruit and experimental conditions 174
8.2.2 Determination of fruit physiological parameters 176
8.2.2.1 Rate of ethylene production 176
8.2.2.2 Rate of respiration 176
8.2.3 Determination of fruit quality parameters 176
8.2.3.1 Physiological loss of weight (PLW) 176
8.2.3.2 Fruit firmness 177
8.2.3.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA 177
8.2.3.4 Individual sugars and organic acids 177
8.2.3.5 Total phenols 178
8.2.3.6 Ascorbic acid 178
8.2.3.7 Total antioxidant capacity 178
8.2.4 Statistical analysis 178
8.3 Results 179
8.3.1 Fruit physiological parameters 179
8.3.1.1 Ethylene (C_2H_4) production 179
8.3.1.2 Respiration (CO_2 production) 179
8.3.2 Fruit quality parameters 182
8.3.2.1 Physiological loss of weight (PLW) 182
8.3.2.2 Fruit firmness 182
8.3.2.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA 182
8.3.2.4 Individual sugars 185
8.3.2.5 Individual organic acids 185

8.3.2.6 Total phenols, ascorbic acid and total antioxidant capacity 187
8.4 Discussion 188
8.5 Conclusions 190
Chapter 9 General discussion, conclusions, limitations and future prospects **191**
9.1 Introduction 191
9.2 General discussions 193
9.2.1 Different formulations of new ethylene antagonist compounds 193
9.2.2 Combined effects of ethylene antagonists and ozone on the cold-stored Granny Smith and Cripps Pink apple fruits 194
9.2.3 Combined effects of ethylene antagonists and AiroFresh® technology on the Cripps Pink fruit stored in controlled atmosphere storage 196
9.2.4 Postharvest fumigation of ethylene antagonists in the Granny Smith apple fruit stored in controlled atmospheric storage 197
9.2.5 Ethylene antagonist treatment in the Gold Rush pear fruit stored in controlled atmospheric storage 198
9.3. Conclusions and recommendations 198
9.4. Limitations 199
9.5. Future prospects 199
Literature cited **201**
Annexure: List of chemicals used for experiment and observations **227**
Statement of contributions **229**

List of figures

Figure 2.1	Top apple fruit producers of the world	8
Figure 2.2	Top pear fruit producers of the world	9
Figure 2.3	The trend of area and production of apples in Australia (1994-2016)	10
Figure 2.4	The trend of area and production of pears in Australia (1994-2016)	11
Figure 2.5	Apple production in Australia by state (2016) (ABS, 2018)	11
Figure 2.6	Pear production in Australia by state (2016) (ABS, 2018)	12
Figure 2.7	Major apple and pear growing regions of Australia	13
Figure 2.8	Apple and pear varieties (A) Granny Smith apple (B) Cripps Pink apple (C) Gold Rush pear	14
Figure 2.9	Structure of ethylene (C_2H_4) molecule	20
Figure 2.10	Reactions in the ethylene biosynthesis pathway (blue boxes), Yang cycle reactions (brown boxes) and key enzymes involved (red thought bubble)	22
Figure 2.11	Ethylene signal transduction pathway	25
Figure 2.12	Inhibition of ethylene biosynthesis at different stages of the biosynthesis pathway	27
Figure 2.13	Structure of 1-methylcyclopropene	29
Figure 2.14	Structure of 1 *H*-cyclopropabenzene and 1 *H*-cyclopropa[*b*]naphthalene	33
Figure 2.15	Structure of ozone (O_3) molecule	38
Figure 3.1	Location of orchards in Western Australia, where the experimental fruit was sourced (A) Curtin Horticulture Research Laboratory, Perth (B) Eastwind Farms, Balingup (C) Newton Brothers Orchards, Manjimup (D) Casuarina Valley Orchards, Beedelup.	43
Figure 3.2	Tinytag *Plus* Gemini Data Loggers used to record temperature and relative humidity data in storage room	47

Figure 3.3	Ozone generators fitted in the cold storage	48
Figure 3.4	(A) Aeroqual IP41 Remote Sensor (B) Aeroqual series 500 monitor	48
Figure 3.5	AiroFresh unit installed in the storage	49
Figure 3.6	(A) Two fruits sealed in a 1L airtight jar with a rubber septum (B) '6890N Network Gas Chromatograph system, Agilent Technology' used to estimate the rate of ethylene production	51
Figure 3.7	Gas chromatographic profiles of ethylene gas production (A) 1.15 ± 0.06 µL L^{-1} ethylene gas standard and (B) gas sample of Granny Smith apple fruit	51
Figure 3.8	'Servomex Gas Analyser, 1450 Food Package Analyser' used to estimate the amount of carbon dioxide evolved	52
Figure 3.9	Servomex Gas Analyser's chromatographic profile of CO_2 standard (8.31±0.17%) and sample gas peaks	53
Figure 3.10	'Texture Analyser' used to determine the fruit flesh firmness	54
Figure 3.11	High-performance liquid chromatography (HPLC) system used for individual sugars and organic acid analysis	56
Figure 3.12	HPLC chromatographic profile of individual standard sugars	58
Figure 3.13	HPLC chromatographic profile of individual standard organic acids	59
Figure 3.14	Flow chart for extraction and estimation of total phenols in pulp	60
Figure 3.15	Flow chart for determination of ascorbic acid in fruit pulp	61
Figure 4.1	Changes in rates of ethylene production due to ethylene antagonist treatments applied as dip and fumigation	71

formulations (T) days past storage (D) in Cripps Pink apple fruit stored for 100 and 150 days. The vertical bars in the graph represent SE of the mean values and are not visible when values are smaller than the symbol. n= 4 replicates (2 fruit per replication). LSD ($P \leq 0.05$) T= 0.10, D= 0.12, TXD= 0.32 for 100 days and T= 0.11, D= 0.13, TXD= 0.34 for 150 days

Figure 5.1 Changes in the rate of ethylene production due to ozone and ethylene antagonist treatment (T) days past storage (D) in Cripps Pink and Granny Smith apple fruits cold-stored for 90 and 120 days. Vertical bars represent SE of mean values and are not visible when values are smaller than the symbol. n=4 replicates (2 fruit per replication). LSD ($P \leq 0.05$) (A) T=0.05, D=0.08, TXD=0.21 (B) T=0.06, D=0.08, TXD=0.21 (C) T=0.11, D=0.14, TXD=0.39 (D) T=0.09, D=0.11, TXD=0.31 97

Figure 6.1 Changes in the rate of ethylene production due to ethylene antagonist fumigation treatment (T) days past storage (D) in Cripps Pink apple fruit stored for 90 and 120 days in CA and ACA storage. Vertical bars represent SE of mean values and are not visible when values are smaller than the symbol. n=4 replicates (2 fruit per replication). LSD ($P \leq 0.05$) T=0.05, D=0.06, TXD=0.17 for 90 days and T=0.04, D=0.05, TXD=0.16 for 120 days 128

Figure 7.1 Changes in the rate of ethylene production due to the ethylene antagonist treatment (T) days past storage (D) in Granny Smith apple fruit stored in CA storage for 90 and 120 days and in Cripps Pink apple fruit stored in CA storage with AiroFresh® for 60 and 150 days. The vertical bars represent SE of mean values and are not visible when values are smaller than the symbol. n=4 154

replicates (2 fruit per replication). LSD ($P \leq 0.05$) (A) T=0.08, D=0.14, TXD=0.28 (B) T=0.07, D=0.11, TXD=0.22 (C) T=0.07, D=0.14, TXD=0.28 (D) T=0.12, D=0.19, TXD=0.39

Figure 8.1 Changes in the rate of ethylene production due to ethylene antagonist fumigation treatment (T) days past storage (D) in Gold Rush pear fruit stored for 150 and 200 days in CA storage. Vertical bars represent SE of mean values and are not visible when values are smaller than the symbol. n=4 replicates (2 fruit per replication). LSD ($P \leq 0.05$) T=0.11, D=0.17, TXD=0.35 for 150 days and T=0.06, D=0.10, TXD=0.19 for 200 days. 181

List of tables

Table 3.1	Source of experiment fruit and locations of orchards	42
Table 3.2	Details of the ethylene antagonists used in different experiments	44
Table 3.3	Details of the columns used in HPLC systems to analyse levels of individual sugars and organic acids	57
Table 3.4	Elution order and retention times of different sugars and organic acids identified using HPLC system	58
Table 4.1	Effect of the different formulations of ethylene antagonists on the climacteric peak onset (days) and peak rate of ethylene (µmol kg^{-1} h^{-1}) and respiration (mmol kg^{-1} h^{-1}) of Cripps Pink apple fruit stored in cold storage	70
Table 4.2	Effect of the different formulations of ethylene antagonists on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Cripps Pink apple fruit stored in cold storage	73
Table 4.3	Effect of the different formulations of ethylene antagonists on the changes in the soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Cripps Pink apple fruit stored in cold storage	75
Table 4.4	Effect of the different formulations of ethylene antagonists on the levels of glucose (g kg^{-1}) and fructose (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in cold storage	77
Table 4.5	Effect of the different formulations of ethylene antagonists on the levels of sucrose (g kg^{-1}) and sorbitol (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in cold storage	78
Table 4.6	Effect of the different formulations of ethylene antagonists on the levels of individual acids (g kg^{-1}) in	79

	the pulp of the Cripps Pink apple fruit stored in cold storage	
Table 4.7	Effect of the different formulations of ethylene antagonists on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of the Cripps Pink apple fruit stored in cold storage	81
Table 5.1	Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0±2°C) with or without ozone gas on the climacteric peak onset (days) and peak rate of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) of the Cripps Pink apple fruit	95
Table 5.2	The climacteric peak onset (days) and peak rates of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) influenced by the ethylene antagonists in the Granny Smith apple fruit stored in cold storage (0±2°C) for 90 and 120 days.	96
Table 5.3	Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0±2°C) with or without ozone gas on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Cripps Pink apple fruit	99
Table 5.4	The physiological loss of weight (PLW) (%) and fruit firmness (N) influenced by the ethylene antagonists in the Granny Smith apple fruit stored in cold storage (0±2°C) for 90 and 120 days	100
Table 5.5	Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0±2°C) with or without ozone gas on the changes in the soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Cripps Pink apple fruit	102
Table 5.6	The soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA influenced by the	103

	ethylene antagonists in the juice of Granny Smith apple fruit stored in cold storage (0±2°C) for 90 and 120 days	
Table 5.7	Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0±2°C) with or without ozone gas on the levels of individual sugars (g kg^{-1}) in the pulp of Cripps Pink apple fruit	106
Table 5.8	The levels of individual sugars (g kg^{-1}) influenced by the ethylene antagonists in the pulp of Granny Smith apple fruit stored in cold storage (0±2°C) for 90 and 120 days	107
Table 5.9	Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0±2°C) with or without ozone gas on the levels of individual organic acids (g kg^{-1}) in the pulp of Cripps Pink apple fruit	108
Table 5.10	The levels of individual organic acids (g kg^{-1}) influenced by the ethylene antagonists in the pulp of Granny Smith apple fruit stored in cold storage (0±2°C) for 90 and 120 days	109
Table 5.11	Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0±2°C) with or without ozone gas on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of Cripps Pink apple fruit	112
Table 5.12	The levels of total phenols, ascorbic acid and total antioxidant capacity influenced by the ethylene antagonists in the pulp of Granny Smith apple fruit stored in cold storage (0±2°C) for 90 and 120 days	113
Table 6.1	Effect of the ethylene antagonists and AiroFresh® on the climacteric peak onset (days) and peak rates of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) of the Cripps Pink apple fruit stored in CA storage for 90 and 120 days	127

Table 6.2	Effect of the ethylene antagonists and AiroFresh® on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Cripps Pink apple fruit stored in CA storage for 90 and 120 days	130
Table 6.3	Effect of the ethylene antagonists and AiroFresh® on the changes in the soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Cripps Pink apple fruit stored in CA storage for 90 and 120 days	132
Table 6.4	Effect of the ethylene antagonists and AiroFresh® on the levels of individual sugars (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in CA storage for 90 and 120 days	134
Table 6.5	Effect of the ethylene antagonists and AiroFresh® on the levels of individual organic acids (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in CA storage for 90 and 120 days	137
Table 6.6	Effect of the ethylene antagonists and AiroFresh® on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of the Cripps Pink apple fruit stored in CA storage for 90 and 120 days	138
Table 7.1	Effect of the ethylene antagonists on the climacteric peak onset (days) and peak rate of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) of Granny Smith apple fruit stored in CA storage	152
Table 7.2	Effect of the ethylene antagonists on the climacteric peak onset (days) and peak rate of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) of Cripps Pink apple fruit stored in CA storage with AiroFresh®	153
Table 7.3	Effect of the ethylene antagonists on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Granny Smith apple fruit stored in CA storage	156

Table 7.4	Effect of the ethylene antagonists on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Cripps Pink apple fruit stored in CA storage with AiroFresh®	157
Table 7.5	Effect of the ethylene antagonists on the changes in the soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Granny Smith apple fruit stored in CA storage	159
Table 7.6	Effect of the ethylene antagonists on the changes in the soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Cripps Pink apple fruit stored in CA storage with AiroFresh®	160
Table 7.7	Effect of the ethylene antagonists on the levels of individual sugars (g kg^{-1}) in the pulp of the Granny Smith apple fruit stored in CA storage	162
Table 7.8	Effect of the ethylene antagonists on the levels of individual sugars (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in CA storage with AiroFresh®	163
Table 7.9	Table 7.9 Effect of the ethylene antagonists on the levels of individual organic acids (g kg^{-1}) in the pulp of the Granny Smith apple fruit stored in CA storage	165
Table 7.10	Table 7.10 Effect of the ethylene antagonists on the levels of malic acid (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in CA storage with AiroFresh®	165
Table 7.11	Table 7.11 Effect of the ethylene antagonists on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of the Granny Smith apple fruit stored in CA storage	167
Table 7.12	Effect of the ethylene antagonists on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of the Cripps Pink apple fruit stored in CA storage with AiroFresh®	168

Table 8.1	Effect of the ethylene antagonists on the climacteric peak onset (days) and peak rate of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) of Gold Rush pear fruit stored in CA storage.	180
Table 8.2	Effect of the ethylene antagonists on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Gold Rush pear fruit stored in CA storage.	183
Table 8.3	Effect of the ethylene antagonists on the changes in the soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Gold Rush pear fruit stored in CA storage	184
Table 8.4	Effect of the ethylene antagonists on the levels of individual sugars (g kg^{-1}) in the pulp of the Gold Rush pear fruit stored in CA storage	186
Table 8.5	Effect of the ethylene antagonists on the levels of individual organic acids (g kg^{-1}) in the pulp of the Gold Rush pear fruit stored in CA storage	187
Table 8.6	Effect of the ethylene antagonists on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of the Gold Rush pear fruit stored in CA storage	188

List of symbols and abbreviations

Abbreviation/ Acronym	Expanded form
®	Registered sign
°C	Degree centigrade
µM	Micromolar
1-MCP	1-Methylcyclopropene
2,5-NBD	2,5 Norbornadiene
Å	Angstrom unit (10^{-8} centimetre)
a.i.	active ingredient
ABA	Abscisic Acid
ABS	Australian Bureau of Statistics
ACC	1-Aminocyclopropane-1-Carboxylic Acid
Acetyl CoA	Acetyl Coenzyme A
ACO	1-Aminocyclopropane-1-Carboxylic Acid Oxidase
ACS	1-Aminocyclopropane-1-Carboxylic Acid Synthase
AIB	A-Amino Isobutyric Acid
AOA	Amino Oxyacetic Acid
APAL	Apple and Pear Australia Limited
ATP	Adenosine Triphosphate
AVG	1-Aminoethoxyvinylglycine
BC	1 *H*-cyclopropabenzene
C_2H_4	Ethylene
CA	Controlled Atmosphere
$CaCl_2$	Calcium Chloride
CO_2	Carbon dioxide
CP	Cyclopropenes
EFE	Ethylene-Forming Enzymes
GA	Gibberellic Acid
GRAS	Generally Recognised as Safe
Ha	Hectare
M	Molarity
Met	Methionine

mM	Milli Molar
mm	Millimetre
MT	Metric Tonnes
MVG	Methoxyvinylglycine
N	Normality
NC	1 *H*-cyclopropa[*b*]naphthalene
nm	Nanometre
O_2	Oxygen
OPP	Oxidative Pentose Phosphate Pathway
PA	Polyamines
PG	Polygalacturonase
PLW	Physiological Loss of Weight
ppm	Parts Per Million
Put	Putrescine
rcf	Relative Centrifugal Force
RP-HPLC	Reverse-Phase High-Performance Liquid Chromatography
rpm	Revolutions Per Minute of Rotor
SAM	*S*-Adenosyl Methionine
Spd	Spermidine
Spm	Spermine
SSC	Soluble Solid Concentration
TA	Titratable Acidity
TCA	Tricarboxylic Acid
TCO	*Trans-c*yclooctene
UNFAO	United Nations Food and Agriculture Organization
USA	The United States of America
USDA	United States Department of Agriculture
USFDA	The United States Food and Drug Administration
UV	Ultra-Violet
WBG	World Bank Group
WRI	World Resources Institute
λ	Wavelength

CHAPTER 1

General introduction

Fruits and vegetables constitute major components of a healthy human diet and are rich in essential vitamins, minerals, dietary fibre and bioactive compounds. They have a significant impact in reducing the several health risks such as cancer, heart diseases and other chronic diseases (Kader, 2001). Apple (*Malus* × *domestica* Borkh.) and European pear (*Pyrus communis* L.) are temperate fruits grown all around the world. Apples and pears are healthy choices for sustained energy as they are free from cholesterol, fat or sodium and a rich source of bioactive compounds as well as the dietary fibres (Skog and Chu, 2003). They are highly relished for their crispiness, sweet taste and typical aroma. Apple and pear are important pome fruits grown in different states of Australia and within Western Australia (WA), they are majorly cultivated in South-Western parts of the WA state. There are several cultivars of apple and pear fruits and the characteristics of each cultivar vary significantly. In Western Australia, Granny Smith, Cripps Pink, Royal Gala and Red Delicious are the most popular cultivars while recently cultivars such as ANABP 01 (WA bred), Scifresh (hybrid from New Zealand) are becoming popular. In the case of pears, Packham's Triumph, Beurré Bosc and Gold Rush are some of the popular cultivars (APAL, 2019).

The rate of postharvest physiological activities (respiration, transpiration and enzyme activities) is very high in the horticulture crops when compared to grain crops and this property makes them highly susceptible to rapid quality deterioration and postharvest losses. The qualitative and quantitative losses in horticultural crops are widely evident at almost every stage of the supply chain (Kader, 2004). It is estimated that nearly 1.3 billion tonnes of food produced for human consumption is either lost or wasted per annum before it reaches the consumer. An estimate of 44% of the total postharvest losses comprise losses in fruit and vegetables (Lipinski et al., 2013). In Australia, the postharvest losses in fruit and vegetables range from 10 to 35 % of the total produced and in monetary terms it is nearly A$ 1,719 million per annum (Lapidge, 2015). One of the main sustainable ways of achieving food security and overcoming the negative impacts caused by postharvest losses at the global level on social, economic and environmental factors, is to bring down these postharvest losses to possibly lowest levels. (Kitinoja et al., 2018). There are several causes for the postharvest losses in the

fruits and vegetables, but it is estimated that nearly 50 % of all the losses are due to the fruit ripening processes promoted by plant hormone ethylene (Blanke, 2014). The fruit ripening process is a genetically controlled natural energy-dependent developmental process in the fruits. During the ripening process, the fruit undergoes several irreversible events at cellular level, which includes break down of cell wall constituents, change in respiration and ethylene production patterns, tissue softening, chlorophyll degradation, loss of water and other reactions which ultimately lead to senescence and deterioration (Taiz et al., 2015). Depending upon the trends of respiration and ethylene production during the ripening process, the fruits are classified into two physiological groups namely climacteric and non-climacteric fruits. The climacteric fruit such as mango (*Mangifera indica* L.), apple and pears exhibit a distinctive rise in the rates of respiration usually coinciding with the onset of the ripening process. This peak is termed as a climacteric peak. The climacteric peak is not evident in the non-climacteric fruits such as grapes (*Vitis vinifera* L.), strawberry (*Fragaria* × *ananassa* Duch.) and sweet orange (*Citrus sinensis* (L.) Osbeck (Biale and Young, 1981).

Fruit ripening is the last stage of the fruit development process. It involves various anabolic and catabolic reactions which result in a series of biochemical, physiological changes in texture, colour, flavour, aroma, and nutritional value (Anwar et al., 2018). Fruit softening during the ripening mainly occurs due to the conversion of starch into sugars, degradation of cell wall materials by hydrolytic enzyme activity and loss of cell turgor due to the water loss through physiological processes (Van Buren, 1979; Giovannoni, 2008). Several aroma volatile compounds are developed during the ripening process, which determine the characteristic smell and taste specific to that of fruit and cultivar (Espino-Díaz et al., 2016). Even though, almost all the changes related to fruit ripening are directly or indirectly promoted by the plant hormone ethylene, not all fruit respond similarly to the ethylene exposure (Anwar et al., 2018). The climacteric fruits such as apples and pears are usually sensitive to ethylene exposure and in response, they accelerate internal ethylene production and other associated ripening changes (Burg and Burg, 1962). Slowing down the ripening process by proper postharvest handling, maintaining optimum storage conditions and regulating ethylene can significantly reduce postharvest losses and maintain optimum fruit quality during storage (Gross et al., 2016).

Modification of storage environments in order to delay the fruit physiological activities is an effective way of extending storage life and maintaining optimum fruit quality during storage (Domínguez et al., 2016). Low-temperature storage or cold storage is an age-old practice to store food commodities for a longer duration. According to the 'Q_{10} temperature coefficient' principle, the storage life of the produce can be doubled with every 10 °C reduction in the storage temperatures, within the optimum temperature limit of a certain fruit (Kitinoja, 2013). The rate of enzyme activity and thus the rate of physiological processes in the fruit is slowed down when stored in low-temperature storage. The fruit stored at low temperatures exhibit reduced respiration, lowered ethylene production, decreased physiological loss of water and effectively maintain the fruit firmness, nutritional and organoleptic properties for an extended period (Paull, 1999; Kitinoja and Kader, 2002; Keller et al., 2013; Gross et al., 2016). The fruit response to the low-temperatures and their safe storage temperatures vary depending upon the species, cultivar, pre- and postharvest handling practices and length of the storage period. Temperate fruits such as apple, pear and cherry can be stored safely at temperatures as low as 0 °C while tropical fruits such as mango, banana and sweet orange develop chilling injury symptoms and the fruit quality deteriorates when storage temperatures are below 5-10 °C (Gross et al., 2016).

Controlled atmosphere (CA) storage constitutes air-tight storage rooms with higher levels of carbon dioxide (CO_2) and lower levels of oxygen (O_2) gases when compared to the normal atmosphere in addition to the lowest possible temperatures and high relative humidity (RH) (Gross et al., 2016). The CA environment retards the rates of respiration and ethylene production as well as delays the ripening-related changes in the fruit, and thus extends its storage life to a considerable extent (Keller et al., 2013). The optimum concentrations of the gases in the CA storage differ with species, cultivar, stage of maturity at harvest and storage temperatures (Gross et al., 2016).

Ozone is a strong oxidising agent and has been popular for its biocidal properties since the early 20th century. It was widely used in purifying the drinking water, milk, meat and alcoholic beverages (Hill and Rice 1982). The water treated with ozone is being used in the horticulture industry to eliminate the microbial population on the fruit surface (Tzortzakis and Chrysargyris 2017). The ozone treatment does not leave any residue on the produce treated as it readily dissociates into oxygen gas. Hence, the ozone was declared GRAS (Generally Recognised as Safe) status by USFDA (United

States Food and Drug Administration) and is also permitted for organic certification in the food industry (Selma et al., 2008). Ozone in the storage environment oxidises the ethylene gas but show very little effect on the internal ethylene production in the fruits (Skog and Chu, 2001). The ozone application significantly affects the storage life and postharvest quality of the fruit, but the positive or negative effects depends upon the ozone concentration, type of the fruit and the storage method (Horvitz and Cantalejo 2014).

AiroFresh® is a relatively new technology and it uses photocatalytic oxidation (PCO) principle to completely oxidise and degrade the airborne volatile organic compounds including ethylene. The technology was developed by Creative Research Technology (CRT), Australia and they claim that these units would induce suitable conditions for maximum efficiency of the ethylene inhibitors in the storage rooms (AiroFresh®, 2019).

Several methods are being used in the horticulture industry to manage the ethylene and thus delay ripening, reduce associated postharvest losses, enhance storage life as well as maintain fruit quality during storage (Gross et al., 2016). The negative effects of ethylene in the fruit can be reduced by inhibition of its biosynthesis or by blocking the action at cellular level. The compounds such as 1-aminoethoxyvinylglycine (AVG), methoxyvinylglycine (MVG) and amino oxyacetic acid (AOA), Cobalt ion (Co^{2+}) and polyamines (spermine, spermidine and putrescine) inhibit ethylene biosynthesis in the plant parts (Abeles et al., 1992). The ethylene action in the fruit is antagonised by the irreversible blocking the ethylene receptor sites and thus preventing the expression of ethylene-responsive genes (Sisler, 2006). Compounds such as cycloalkenes, terpenes and cyclopropenes were identified to block ethylene receptors in the fruit but their effectiveness in antagonising the ethylene action varied widely (Sisler et al., 2006). 1-alkyl cyclopropenes are effective ethylene inhibitors and unlike other compounds, their antagonistic effect persists for a longer period even with a single exposure (Sisler et al., 2003; Apelbaum et al., 2008). In comparison to all other cyclopropenes (CP) identified, 1-methylcyclopropene (1-MCP) is the most effective ethylene antagonist and stable CP at the gaseous stage (Sisler et al., 2006). The commercial formulations of 1-MCP are widely being used in the horticulture industry to extend the storage life of different horticulture crops (Valero et al., 2016). 1-MCP effectively antagonised ethylene action in different fruit and vegetables but the varied responses have been

reported by different researchers for the effects of 1-MCP treatments on nutritional quality, levels of sugars and acids (Watkins, 2006). The effect of 1-MCP on the fruit physiology and quality depends upon the species, cultivar, maturity stage and storage conditions (Huber et al., 2003).

Several researchers have reported the effectiveness of 1-MCP in antagonising the ethylene action in the fruit and vegetables, yet it still has some limitations. The 1-MCP boils at the temperatures as low as 0 °C and is unstable at room temperature in the liquid state (Sisler et al., 2006). It is available only as an expensive service but not as a product making it unaffordable to many fruit growers. There is a need to explore the possibilities to develop alternative compounds which could antagonise the ethylene action as good as 1-MCP and also address the limitations of 1-MCP. Two potential ethylene antagonist compounds 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa [*b*]naphthalene (NC) were formulated by Singh et al. (2018) which possess the capacity to inhibit a range of ethylene responses in the fruit and maintain commercial fruit quality during storage. The proposed mode of action of these compounds is similar to that of 1-MCP (Musa, 2016).

The combination of cold and CA storage conditions with ethylene antagonist treatments such as 1-MCP exhibited synergistic relationship in improving storage life and in maintaining the fruit quality during storage (Watkins, 2006). Liew and Prange (1994) recorded enhanced concentrations of residual ozone and higher effectiveness of ozone when applied in low-temperature storage environments. Bai et al. (2009) reported that the pome fruits stored in CA storage exhibit sudden rise in ethylene production when taken out of the storage. The application of ethylene antagonists along with CA storage maintained the rate of ethylene production low even after removing from the storage (Watkins, 2006; Mattheis, 2008; Bai et al., 2009).

There is no research reported on the combined effects of the ethylene antagonists (BC, NC and 1-MCP) and cold or CA storage as well as with ozone or AiroFresh® on the rates of respiration and ethylene production and on the postharvest quality of apple and pear fruits during storage. The combined effect of the new ethylene antagonist compounds and different storage environments could effectively inhibit the ethylene action and retard autocatalytic ethylene production in fruit as well as maintain optimum quality. The research project was developed after series of consultations with

Western Australian fruit growers. All the experiments were conducted under standard storage conditions used by the grower, for the results to be more relevant and applicable to them.

General objective:

To investigate the effect of BC, NC and 1-MCP in retarding the rates of respiration and ethylene production, reducing PLW and in maintaining fruit firmness, levels of bioactive compounds and other fruit quality parameters of Granny Smith apple, Cripps Pink apple and Gold Rush pear fruits stored in cold or CA with or without ozone or AiroFresh®.

Specific objectives:

1. To study the effects of different formulations of new ethylene antagonist compounds on the ethylene production and fruit quality of the cold-stored Cripps Pink apple fruit.
2. To investigate the combined effects of ozone as well as BC, NC and 1-MCP in regulating ethylene production and maintaining fruit quality of Cripps Pink and Granny Smith apples stored in cold storage.
3. To evaluate combined effects of BC, NC and 1-MCP as well as AiroFresh® technology on the rates of respiration, ethylene production and the fruit quality of Cripps Pink apple stored in controlled atmospheric storage.
4. To study the effect of the postharvest fumigation of new ethylene antagonists and 1-MCP on ethylene production and fruit quality of Granny Smith apples stored in controlled atmospheric storage.
5. To investigate the effect of BC, NC and 1-MCP in antagonising the ethylene effects and maintaining fruit quality in Gold Rush pear stored in controlled atmospheric storage.

CHAPTER 2

General review of literature

2.1 Introduction

Fruit and vegetables are considered as an important constituent of a healthy diet. They form two out of five core food groups of balanced diet recommended by the Australian Dietary Guidelines (2013). These are relished for their unique tastes and flavours combined with a number of health and healing properties. Fruits are a rich source of vitamins, minerals and antioxidants. The flavonoids in apple fruit are proved to have cardioprotective effects by enhancing endothelial functioning as well as arterial stiffness (Bondonno et al., 2018).

Apple (*Malus* × *domestica* Borkh.) and European pear (*Pyrus communis* L.) are the pome fruits belonging to the rose family, *Rosaceae* and sub-family *Pomoideae*. These fruits are characterised by the tough membranous core enclosing several small seeds. The core is encased in by the fleshy edible thalamus. These trees are grown mostly in a temperate climate and are usually deciduous with a distinct period of dormancy during the winter season. The buds require chilling temperatures for a certain period to break bud dormancy and bloom in the spring. Pome fruits are usually grown from spring blossom and the harvest starts from the late summer and continues till the late autumn season.

Apples and pears are the main pome fruits grown in Western Australia and other parts of the country, followed by Nashi (*Pyrus pyrifolia* Nakai.) and quince (*Cydonia oblonga* Miller.). Apple is believed to have originated within the region of Asia Minor (Juniper et al., 1998). The pears are believed to be originated from three different centres of diversity (Bell et al., 1996). *Pyrus pyrifolia* and *P. ussuriensis* from China; *P. communis* from the Caucasus Mountains and Asia Minor and *P. communis* and other natural hybrids from Central Asian centre (Dondini and Sansavini, 2012). Apples and pears are rich in carbohydrates, phenolic compounds, pectin and nearly no cholesterol, fat or sodium (Skog and Chu, 2003). It is estimated that one serving (150g) of apple fruit provide up to 8 % ascorbic acid and 2 % of vitamin A and iron of the daily adult requirement (Skog and Chu, 2003). There are more than 7000 apple varieties worldwide, but only a few of them are commercially cultivated (Naeve, 1997).

Pear fruit flesh is usually firmer than apples but turns to melting-soft as ripening progresses due to pectin hydrolysis (Blattný, 2003). Characteristics of the fruit vary significantly with the variety and thus, varietal selection by the growers is done carefully according to consumers' preference. The apple and pear fruit varieties grown in Western Australia are available all year round because of the different storage technologies and their relatively high storage capacity, when compared to other fruit.

2.2 Economic importance of apples and pears

2.2.1 World statistics

Apples and pears are highly relished fruits in the world and grown globally in about 94 and 85 countries, respectively. During 2016, it was estimated that apples and pears were being cultivated in an area of 5.29 and 1.59 million hectares (ha), respectively, yielding 89.33 million metric tonnes (MT) of apple and 27.35 million MT of pear fruits worldwide. Mainland China is the highest pome fruit producer and it alone produced about 30 % of the world's apple and 43 % of the world's pear fruits (Figures 2.1 and 2.2) during 2016 (FAOSTAT, 2018).

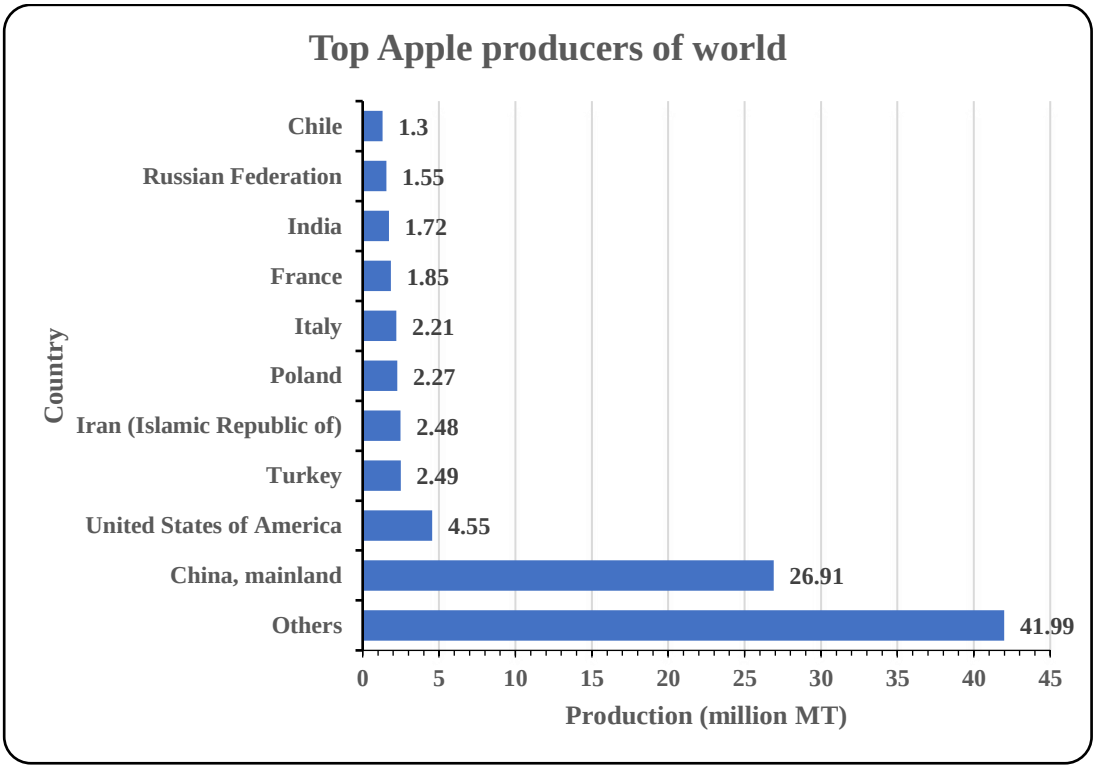


Figure 2.1 Top apple fruit producers of the world (FAOSTAT, 2018)

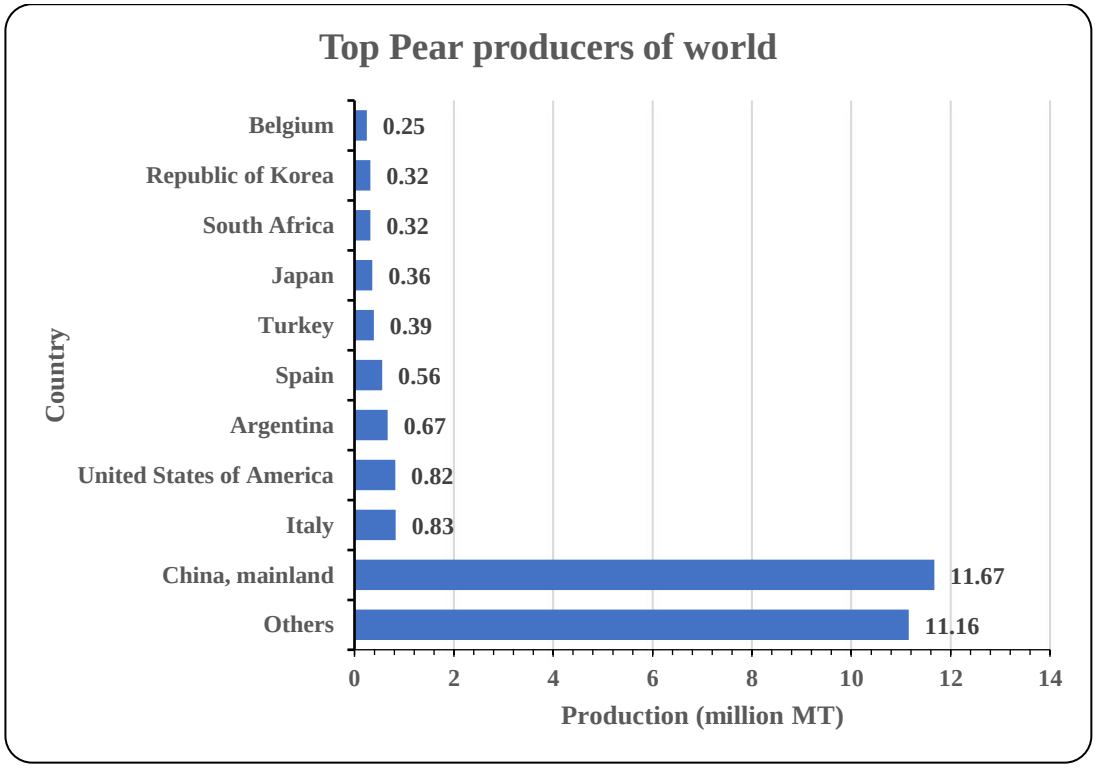


Figure 2.2 Top pear fruit producers of the world (FAOSTAT, 2018)

The apple and pear fruits claim high global export market, valuing US$ 7.27 billion and US$ 2.45 billion, respectively, by the export of 9.04 million MT of apple and 2.72 million MT. The United States of America (USA), France, Italy, Mainland China and Chile are the top apple fruit exporting countries and constitute one-third of total world apple exports. Argentina exports highest quantity (0.34 million MT) of pear fruit followed by Mainland China (0.28 million MT), Belgium (0.25 million MT), the Netherlands (0.24 million MT) and USA (0.17 million MT) (FAOSTAT, 2018).

The worldwide import market of the apple and pear fruit valued US$ 7.80 billion and US$ 2.58 billion respectively, with the total global imports of 8.90 million MT of apple and 2.70 million MT of pear. The Russian Federation, Germany, United Kingdom and the Netherlands import major share of the apple and pears in the world. Together these countries imported 2.16 million MT apples and 0.66 million MT pears fruits during 2016 (FAOSTAT, 2018).

2.2.2 Australian statistics

Australia constitutes a very small share of the world pome fruit production. The area under apple cultivation and the quantity of the fruit produced in Australia had an unsteady trend from 1994 to 2016 (Figure 2.3). In Australia, during 2016 about 0.31 million MT of the apple fruit was harvested from 19,212 ha crop area, which is the same as the quantity of the fruit harvested during 1994 (FAOSTAT, 2018).

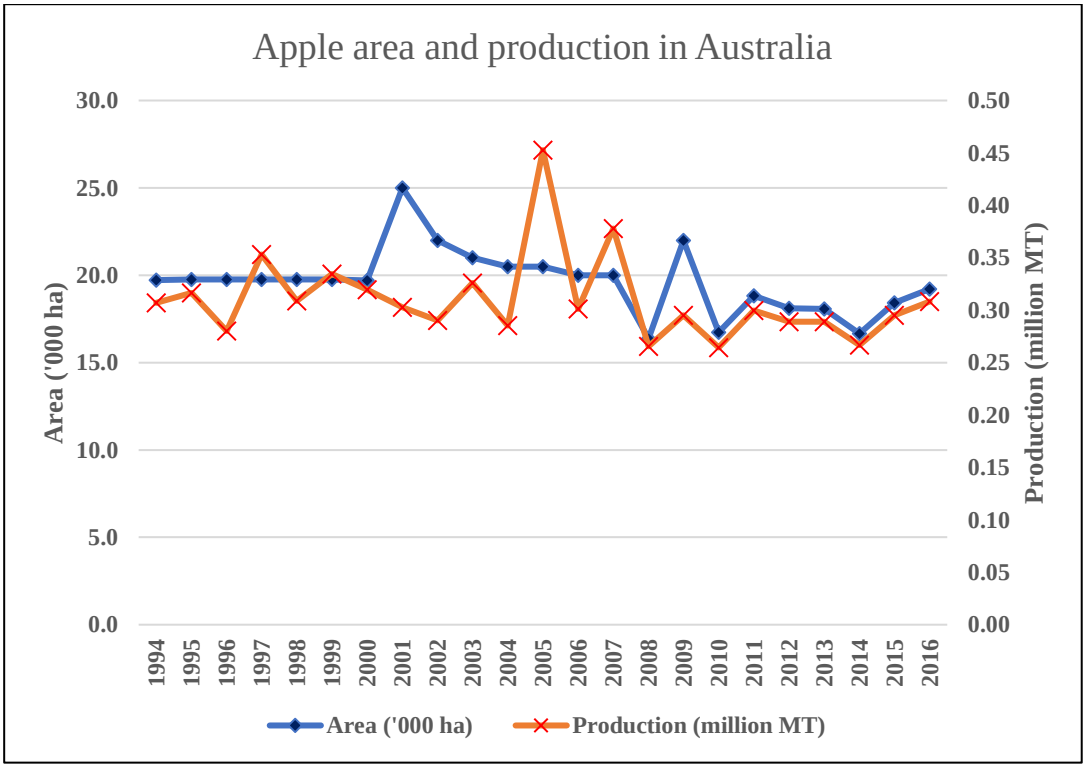


Figure 2.3 The trend of area and production of apples in Australia (1994-2016) (FAOSTAT, 2018)

In Australia, the pear fruit production and area under cultivation has decreased from 1994 to 2016 (Figure 2.4). During 1994, about 0.16 million MT of pear fruit was produced from 7,530 ha crop area, which decreased to 0.10 million MT production and 5,730 ha crop area by 2016 (FAOSTAT, 2018). There is decline in number of pear growers as most of the small scale growers are leaving industry due to different reasons (AgriFutures, 2019).

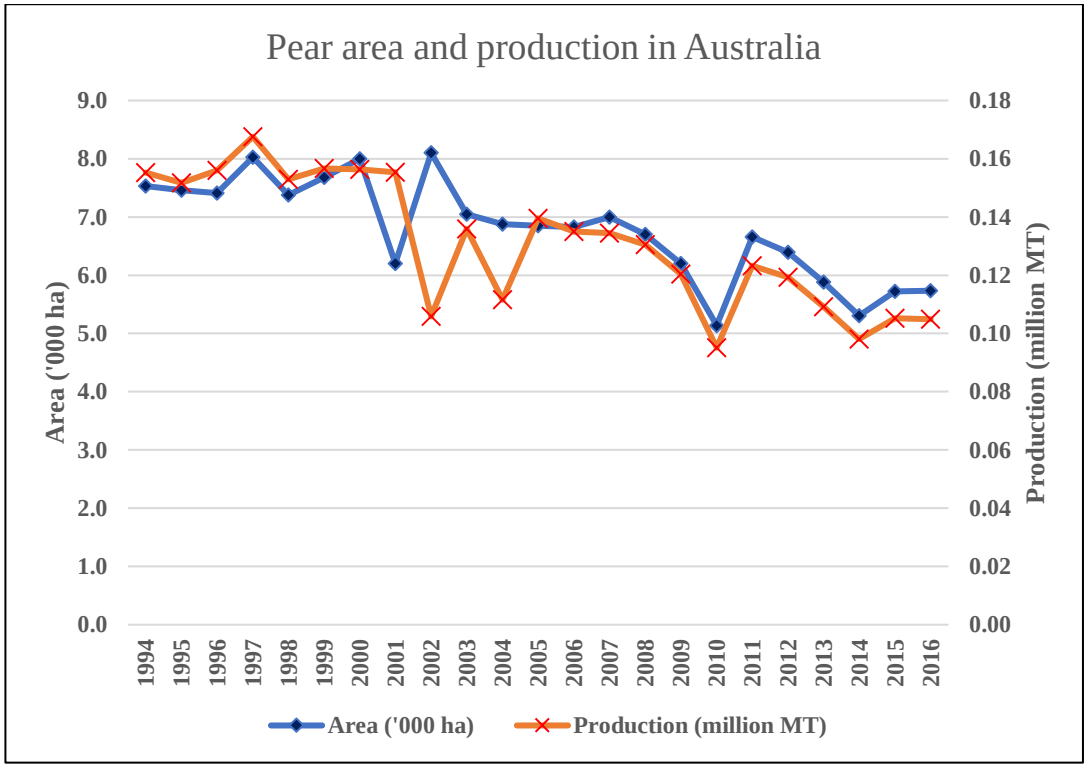


Figure 2.4 The trend of area and production of pears in Australia (1994-2016) (FAOSTAT, 2018)

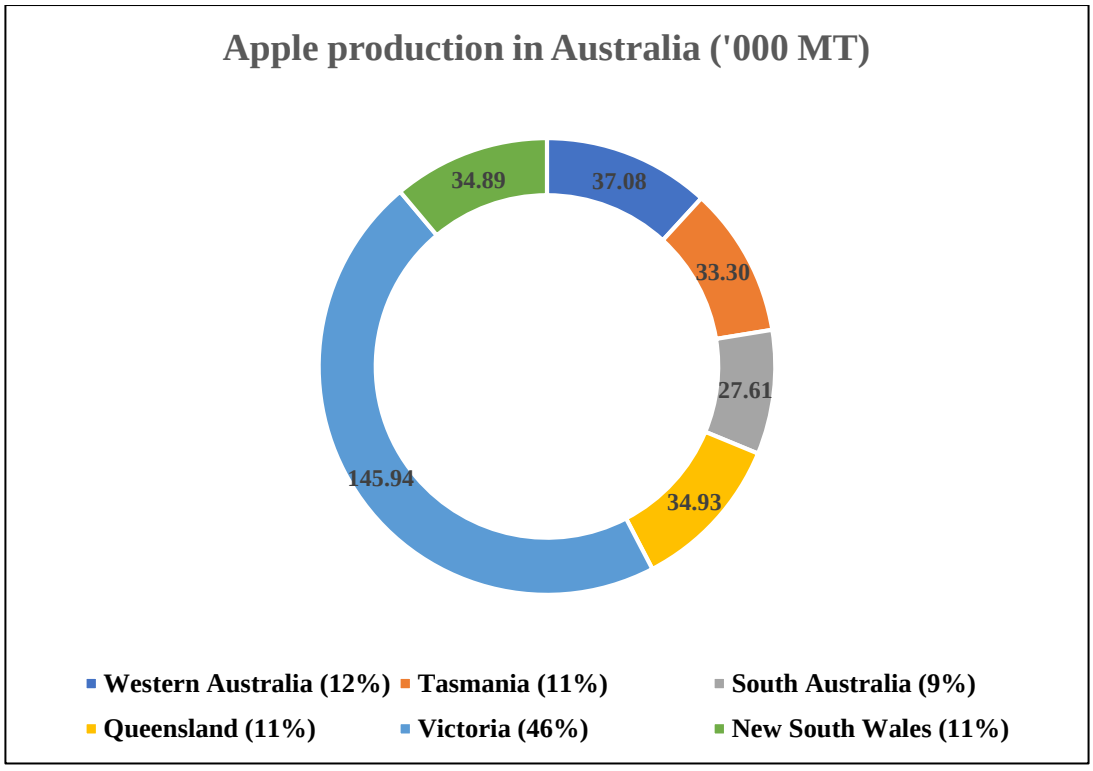


Figure 2.5 Apple production in Australia by state (2016) (ABS, 2018)

In Australia, the state of Victoria produces a major share of pome fruits, about 46 % of total apple and 87.6 % of total pear fruit produced during 2016-17 (Figure 2.5 and 2.6) (ABS, 2018). Western Australia constitutes 12 % of the total apple and 3.8 % of total pear fruit produced in the country (ABS, 2018). Most of the apple and pear orchards in Western Australia are located in areas of Manjimup, Donnybrook, Pickering Brook and Perth Hills regions (Figure 2.7).

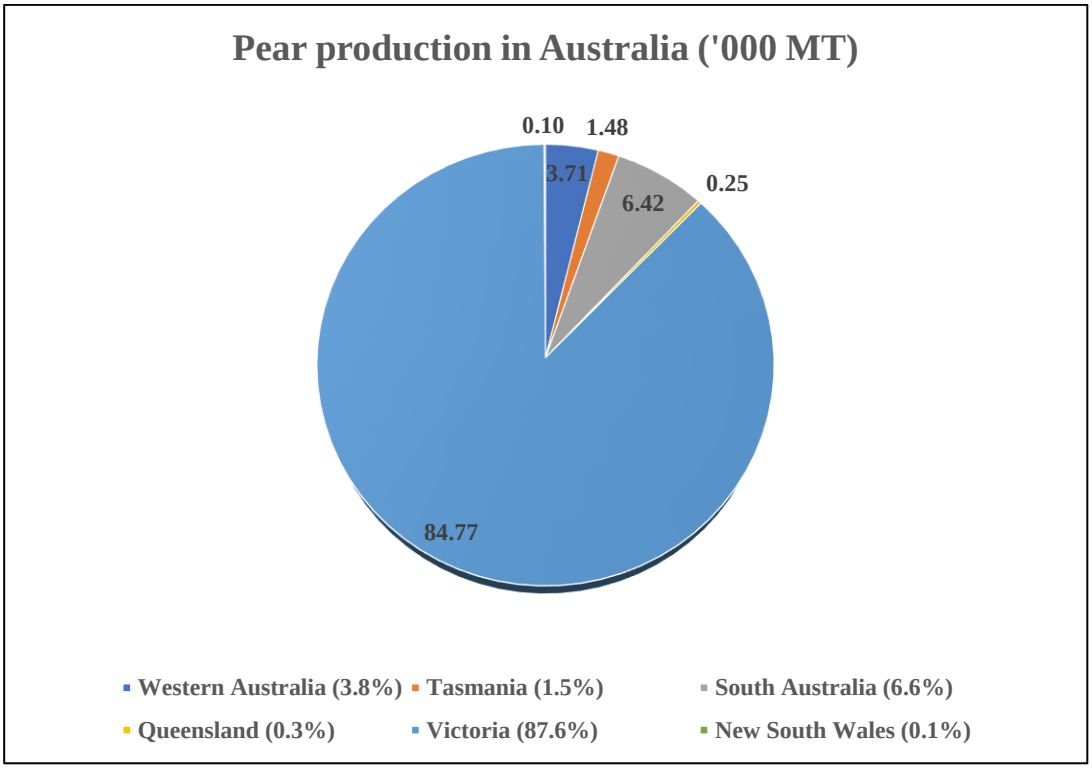


Figure 2.6 Pear production in Australia by state (2016) (ABS, 2018)

2.2.3 Major commercial cultivars of apple and pear

Australia produces a large range of apple and pear varieties. Granny Smith (Figure 2.8A), Cripps Pink (Figure 2.8B), Fuji, Red Delicious and Royal Gala are most popular apple varieties and some of the new varieties such as The sentence has been replaced mentioning ‘ANABP 01 (WA bred), Scifresh (hybrid from New Zealand)’ are gaining popularity in recent times (APAL, 2019). Beurré Bosc, Gold Rush (Figure 2.8C), Corella Forelle, Josephine de Malines, Packham’s Triumph, Red Anjou, Red Sensation, Williams’ Bon Chretien and Winter Nelis are main types of pear cultivars grown in Australia. Packham’s Triumph pears are the most popular among them as fresh fruit and main exported pear variety (APAL, 2019).

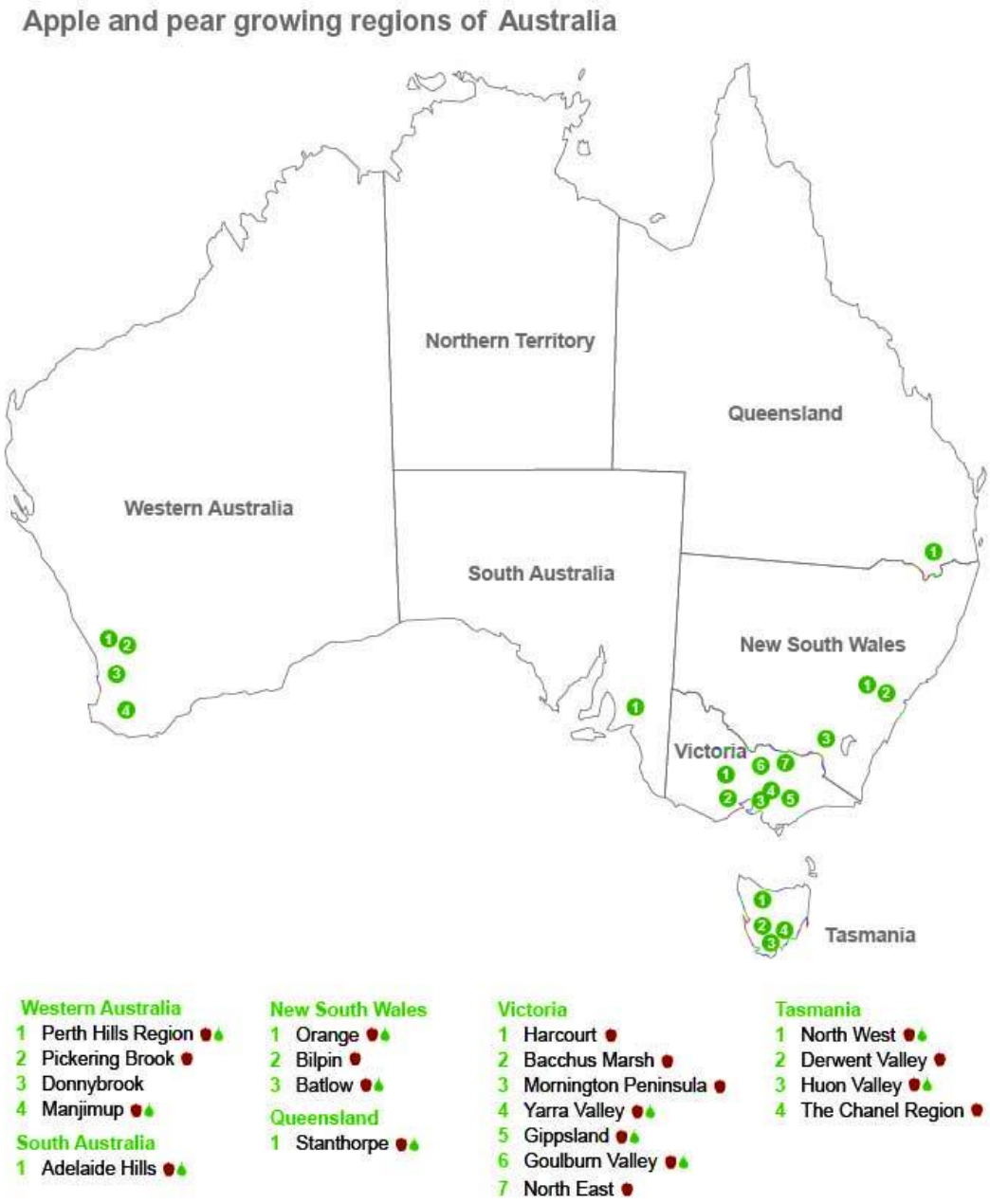


Figure 2.7 Major apple and pear growing regions of Australia (APAL, 2019).

Source: http://apal.org.au/industry-info/growing-regions/

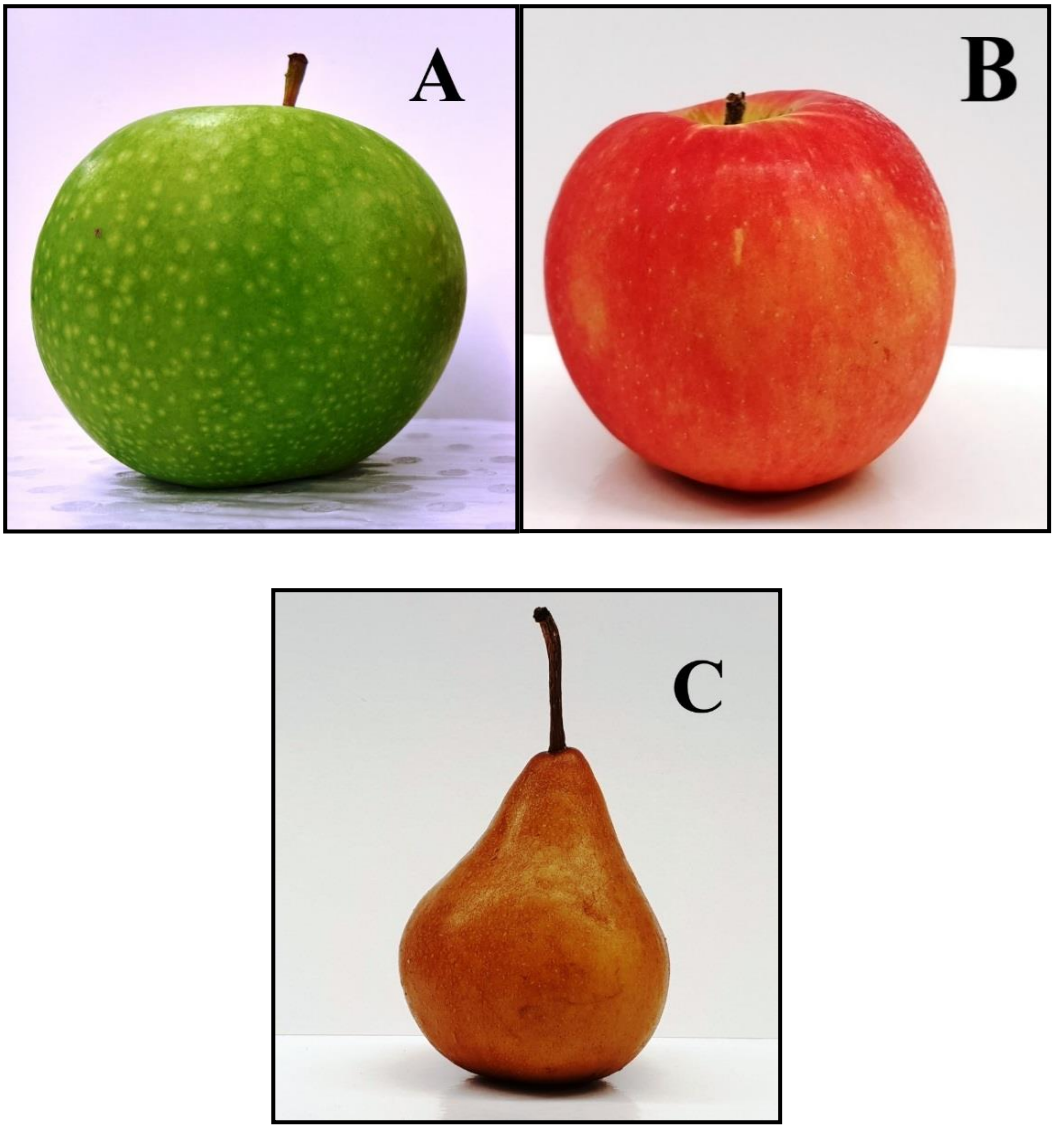


Figure 2.8 Apple and pear varieties (A) Granny Smith apple (B) Cripps Pink apple (C) Gold Rush pear

2.3 Postharvest losses

The issue of global food security and the prospect of necessity to feed a rapidly growing population at a reasonable price have now emerged as an important societal concern. Studies conducted by the United Nations Food and Agriculture Organization (UNFAO), World Resources Institute (WRI) and the World Bank Group (WBG) have indicated that global postharvest losses are substantial and widely quoted that an estimate of one-third of all the agricultural produce by weight, approximately 1.3 billion tonnes, is being lost and does not reach consumers (Lipinski et al. 2013). Both qualitative and quantitative losses are significantly evident in horticultural crops from

harvest to consumption. It is estimated that 44 % of the total postharvest losses constitute fruit and vegetable losses (Lipinski et al. 2013). Postharvest food losses have several negative impacts on the world economy, environment and sociological factors. Prevention of these postharvest losses is being increasingly argued as sustainable means to ensure food security (Kitinoja et al. 2018). In an economic perspective, the food lost represents the wasted investment that would ultimately reduce the grower's income and further increase consumers' expenses. Environmentally, postharvest losses imply wastage of precious natural resources used for the production; including water, land, other non-renewable resources and undesirable greenhouse gas emissions connected with the usage of the fossil fuels. From the social aspect, reducing losses would assist in feeding the undernourished population, which was estimated to be nearly 795 million (Porat et al., 2018). Several factors are associated with postharvest losses in fruits and vegetables which includes improper postharvest handling, fungal diseases, storage conditions and majorly the ripening linked physiological activities, such as rate of respiration and ethylene production (Tavallali and Moghadam, 2015). Fruit ripening is a natural process and is also an inherent cause for increased vulnerability to the postharvest losses in the fruits. It is essential to understand the appropriate physiological and environmental factors promoting postharvest deterioration to design relevant strategies to reduce the postharvest losses and maintain the quality and safety of horticultural commodities (Kader 2004). Slowing down the ripening process can significantly contribute to the reduction of postharvest losses. Blanke (2014) mentioned that approximately 50 % of all the postharvest losses in horticultural crops are directly or indirectly due to natural ripening gas hormone ethylene. It was also suggested that the regulation of the ethylene is one of the possible solutions to reduce food losses by controlling ripening and retarding senescence processes in the fruit and vegetables at different stages of the food supply chain. Ethylene is an odourless and colourless gaseous compound present in nature and also produced by fruit and vegetables as they proceed with the ripening process (Saltveit, 1999). Ethylene gas induces and accelerates irreversible physiological changes such as ripening, senescence and ultimately deterioration in case of horticulture produce. The amount of ethylene gas emitted and levels of the response of the produce to ethylene hugely vary, depending upon the genetic and physiological factors (Saltveit, 1999).

2.4 Fruit ripening

Most of the fruits are attractive to the consumer due to their unique aesthetic qualities such as colour, flavour and texture. During the fruit development and maturation, the photosynthates from different plant parts are directed to the fruit organs followed by the development of aroma compounds and pigments inducing different colours. The fruit ripening is an irreversible degradative process characterised by different biochemical, physiological and structural changes and is considered as the last stage of fruit development. This process involves modification of the fruit making it suitable for seed dispersal or to make it attractive for organisms to facilitate seed dispersal process (Giovannoni, 2004). Such modifications typically involve hydrolysis of starch, sugar accumulation, fruit softening due to enzymatic breakdown of the cell walls, reduction in organic acids, tannins and other phenolic compounds. Accumulation of anthocyanins, carotenoids and other pigment molecules in fruit epidermis induce bright colours and enhance visibility to animals allowing seed dispersal by ingestion (Giovannoni, 2001). The fruit ripening process also involves significant changes in patterns of respiration process and levels of different growth regulators especially ethylene. It is usually followed by the senescence process and then results in tissue decay, deterioration and death (Tucker, 2012).

2.4.1 Respiration

Fruit ripening is a complex process and includes several anabolic and catabolic events. Primarily, it involves the synthesis of enzymes, hormones, pigments and flavour compounds and all these activities require energy. These energy needs are met by the breakdown of respiratory substrates (Giovannoni, 2008). The sugars and organic acids are major respiratory substrates in most of the fruits. The respiratory quotient (ratio of the volume of carbon dioxide evolved to the volume of oxygen consumed during respiration) value of most of the fruit suggests that sugars are main respiratory substrates in fruit (Tucker, 2012).

Sugars are oxidised during respiration process to release energy in the form of adenosine triphosphate (ATP). In plants, oxidation of sugars commonly occurs

through respiratory pathways namely glycolysis (conversion of glucose to pyruvate) and oxidative pentose phosphate pathway (OPP) (parallel pathway to glycolysis). A significant proportion of organic acid breakdown was also reported in several instances during respiration (Tucker, 2012). Malic acid is one of the major organic acid respiratory substrates in the plants. It is converted to pyruvate in the presence of a malic enzyme (El-Shora and ApRees, 1991). The pyruvates formed from glycolysis, OPP and malate metabolism are oxidised through tricarboxylic acid (TCA) pathway in the plant mitochondria to form ATP, the energy units of a plant cell, are formed via electron transport chain (Tucker, 2012).

Based on the patterns and rates of respiration activity during the ripening process, all the fruits are broadly classified into two physiological categories - climacteric and non-climacteric fruits. Climacteric fruits such as apple, pear, mango (*Mangifera indica* L.) and avocado (*Persea americana* Mill.) exhibit characteristic rise in respiration rates during ripening. This typical rise in respiration rate is termed as 'respiratory climacteric'. In case of the non-climacteric fruits such as grapes (*Vitis vinifera* L.), strawberry (*Fragaria* × *ananassa* Duch.) and sweet orange (*Citrus sinensis* (L.) Osbeck), the respiration rates do not show any peak, but may reduce gradually during the ripening process (Biale and Young, 1981). The respiratory climacteric is generally observed just before ripening initiation in fruits, but the exact role of a sharp rise in the respiration rate in the fruit ripening process is still unclear. It was assumed that the increased levels of respiration activity produce higher amounts of ATP essential for ripening-related activities such as synthesis of nucleic acids, amino acids, pigment molecules and starch breakdown. However, Solomos (1983) calculated the net energy requirements for the ripening process and found that the energy required was much lesser when compared to the net energy generated during the respiratory climacteric. Tucker (2012) suggested that the respiratory climacteric rise might be just a response to a hike in ethylene biosynthesis in climacteric fruit. This also explains low respiration rates in non-climacteric fruits, where relatively low levels of ethylene are produced during ripening.

2.4.2 Fruit colour

Colour change in different parts of fruit is a common phenomenon associated with fruit ripening. However, not all the fruits show colour changes, for example, Granny

Smith variety of apple remain green colour even at commercial maturity. In general, the colour changes in fruits are due to the degradation of chlorophyll pigments and biosynthesis of one or more colouring pigments such as carotenoids and anthocyanins (benzopyran derivatives) (Daun, 2005). The carotenoid pigments are widely distributed in the plant kingdom and are responsible for a spectrum of yellow, orange and red colours (Priyadarshini, 2017), while anthocyanins individually or along with other polyphenols contribute to a range of colours from red to blue (Daun, 2005). Carotenoids are terpenoid compounds extensively synthesised during ripening process from acetyl CoA through the mevalonic acid pathway. The mevalonic acid pathway is essential during fruit ripening processes and is also responsible for the synthesis of abscisic acid (ABA) and gibberellins (Tucker, 2012). Anthocyanins are a wide range of pigments located in plant vacuoles generally derived from flavonoid compounds. Cyanidin-3-galactoside is very common anthocyanin responsible for peel and pulp colour in fruits like apple, plum (*Prunus salicina* L.) and blueberries (*Vaccinium corymbosum* L.) (Tucker, 2012). Accumulation of anthocyanins during fruit ripening process is believed to be regulated by the ethylene hormone, which in turn control several other aspects of ripening (Tucker, 2012).

2.4.3 Flavour development

Development of distinctive flavour is a vital change during the fruit ripening. Fruit flavour depends on the complex interaction between sugars, organic acids, tannins, phenols and different aroma volatiles. The profile and proportion of these constituents change with the maturation (Fellman et al., 2000). The sugars and organic acids primarily contribute to fruit taste, while astringency in some fruits is derived from phenols and tannins. Several fruit-specific aroma volatiles are responsible for the development of characteristic fruit smell (Espino-Díaz et al., 2016). Aroma volatile compounds are synthesised as a result of fatty acid, nucleic acid and carbohydrate metabolism. Synthesis of aroma volatile compounds is initiated and regulated by the plant hormone ethylene (Fellman et al., 2000). Aldehydes form chief aroma compounds at the pre-climacteric stage of pome fruits, followed by a significant increase in alcohol levels with the advancement of maturation and finally esters dominate as chief aroma volatiles at the ripe stage of fruit (Suwanagul and Richardson, 1998; Fellman et al., 2000).

2.4.4 Fruit softening

Fruit ripening and fruit softening processes are usually interconnected and determine the quality and storage life of the fruit. Fruit softening occurs due to three different mechanisms: starch degradation, loss of cell turgor and cell wall metabolism. These processes can occur either in independent or interdependent ways with other fruit physiological activities (Van Buren, 1979; Giovannoni, 2008). Breakdown of starch during ripening process results in significant textural changes, particularly in starch-rich fruits like banana (*Musa* spp.), where starch constitutes a high percentage of the fruit fresh weight (Tucker, 2012). Water status in the cell is also one of the essential determinants of fruit firmness. Loss of water causes loss of turgor in plant cells, resulting in fruit shrivelling and ultimately reduction in fruit firmness (Harker et al., 1997). Loss of water from the fruit can be due to physical factors such as a rise in surrounding temperatures or due to physiological factors such as transpiration. Increase in respiration rates during ripening process tends to increase the temperature in the fruit, which in turn increase water loss from the fruit due to transpiration (Becker and Fricke, 1996). Out of all the mechanisms, cell wall degradation is considered primarily responsible for the fruit textural changes and fruit softening. The cell wall is a composite structure, which induces shape and rigidity to the cells. It is made up of network cellulose microfibrils surrounded by the hemicellulose matrix. The pectin network binds the cells to each other and maintains the fruit texture. Fruit ripening involves enzyme-mediated hydrolysis of cell wall materials and these changes in cell wall ultrastructure result in fruit softening (Cosgrove, 2001). Hydrolysis of these cell wall constituents break cell-cell adhesion by depolymerisation of pectin fibres and further increase cell porosity and make cell wall flexible (Brummell and Harpster, 2001). Earlier, polygalacturonase (PG) enzyme alone was believed to be the cause for the majority of cell wall degradation activities and fruit softening (Bennett and Della-Penna, 1987), until PG antisense experiments ascertained the involvement of several other enzymes and complex interconnected phenomenon (Carrington et al., 1993). Exo-polygalacturonase (EC 3.2.1.67), Endo-polygalacturonase (EC 3.2.1.15), Pectin methylesterase (PME) (EC 3.1.1.11), β- galactosidase (EC 3.2.1.23), α-L-arabinofuranosidase (EC 3.2.1.55) are some of the key cell wall modifying enzymes identified in apple fruit (Johnston et al., 2002). Involvement of plant hormone ethylene

in the activation of these cell wall hydrolysing enzymes has been well postulated (Prasanna et al., 2007; Giovannoni, 2008).

2.5 Ethylene

Ethylene (ethene) is the simplest unsaturated hydrocarbon and first known gaseous plant hormone (Yang and Hoffman, 1984). It is a symmetrical molecule with two carbon atoms bound by one double covalent bond and four hydrogen atoms bound to carbon atoms by a single covalent bond (Figure 2.9). It is flammable, colourless lighter than air and undergoes oxidation readily. Ethylene is widely present in the atmosphere. It is released not only by plants and micro-organisms but also by non-living things like rubber (Jacobsen and McGlasson, 1970), lacquer (Wills and Patterson, 1970) and by burning hydrocarbon fuels (Müller et al., 1997).

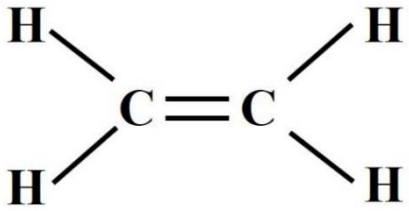


Figure 2.9 Structure of ethylene (C_2H_4) molecule

2.5.1 History

In the early 19th century, it was observed that trees close to the street lamps lit by coal gas showed extensive defoliation when compared to other trees. Later, Dimitry Neljubov (1901), observed an abnormal growth in pea (*Pisum sativum* L.) seedlings grown in dark and identified that ethylene molecule present in the lab air from coal gas, caused the response, which was later termed 'triple response'. In 1934, R. Gane and his group discovered ethylene as a product of natural plant metabolism, responsible for a range of effects in plants even at very low concentrations and hence classified it as a hormone (Taiz et al., 2015). Till late 20th century, ethylene was not considered as important plant hormones and many plant physiologists believed that ethylene only had an indirect or insignificant role in plant physiological activities. With the advent of gas chromatographic techniques, for first-time ethylene gas in

plants could be quantified to physiologically significant levels. Burg and Thimann (1959) then discovered the essential role of ethylene as a plant growth regulator using gas chromatography techniques.

2.5.2 Biochemistry of ethylene biosynthetic pathway

Ethylene is synthesised by all the plants, with amounts varying by more than hundred-fold even between different life stages and is determined by several biotic and abiotic factors (Grierson, 1987). The speed of ethylene biosynthesis pathway studies was hampered for a long time even after its discovery, due to lack of suitable extraction and quantification techniques. Unlike other plant hormones, ethylene is a gaseous hormone and is not convenient to extract by homogenising the ethylene-producing tissues. With the invention of gas chromatography, different studies were then carried out using plant tissues, ripe fruits, senescing tissues, wounded plant tissues and found that ethylene is derived from the precursor methionine. Following this discovery, the rapid progress in ethylene biosynthesis pathway studies was well elucidated by Burg and Burg (1962), Abeles (1973), Lieberman (1979) and Yang and Hoffman (1984).

The biosynthesis of ethylene in higher plants from methionine involves three key reactions: (*i*) Conversion of methionine (Met) into *S*-adenosyl methionine (SAM) catalysed by *SAM synthetase* enzyme (*ii*) Formation of 1-aminocyclopropane-1-carboxylic acid (ACC) from SAM catalysed *ACC synthase* (ACS) enzyme (*iii*) oxidation of ACC to form ethylene catalysed by *ACC oxidase* (ACO) enzyme (Figure 2.10). The three enzymes catalysing these key reactions were earlier commonly referred to as ethylene-forming enzymes (EFE) (Imaseki, 1991), and later renamed after the discovery of oxygen-requiring enzymatic mechanism (Grierson, 2012).

As discussed above, methionine is the precursor and the main component for ethylene biosynthesis in plants, but the levels of methionine in fruits like apple is very low to compensate even normal rates of ethylene production. Hence, Baur and Yang (1972) suggested a mechanism, where methionine is recycled continuously during ethylene production to sustain high ethylene production in apple fruit. In a subsequent publication, Adams and Yang (1977) explained that SAM gets recycled to form methionine again. They demonstrated that SAM gets converted into 5'-methylthioadenosine (MTA), which is rapidly hydrolysed to form 5'-methylthioribose

(MTR). MTR further receive one phosphate from ATP to form 5'-methylthioribose-1-phosphate (MTR-1-P), which is then converted to α-keto-γ-methylthiobutyric acid (KMB). Finally, KMB is converted into methionine by transamination process. This cycle of conversion of SAM back to methionine through MTA, MTR and KMB is called as 'Yang cycle' (Figure 2.10).

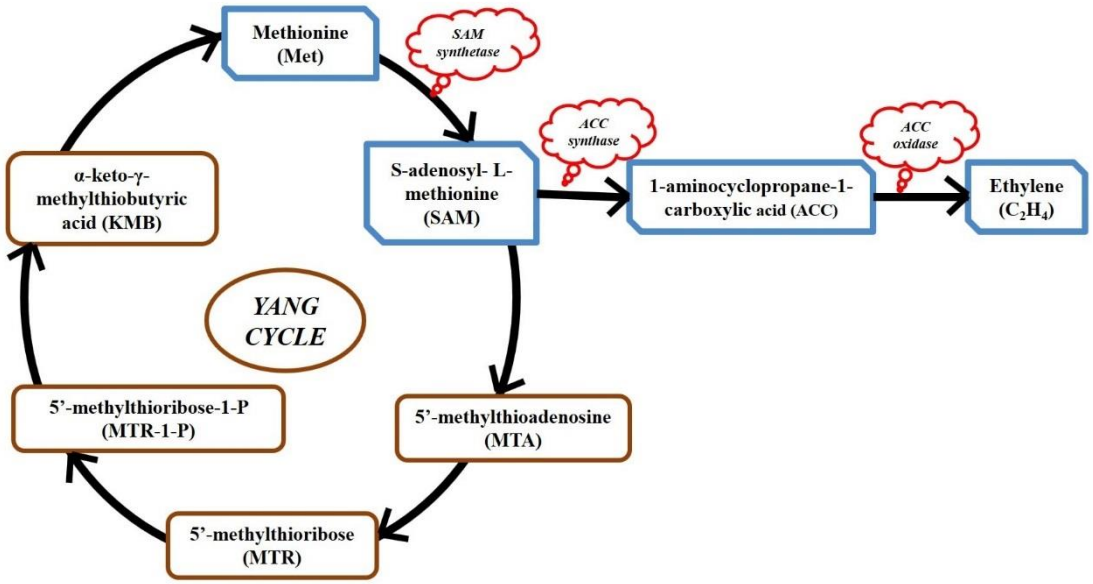


Figure 2.10 Reactions in the ethylene biosynthesis pathway (blue boxes), Yang cycle reactions (brown boxes) and key enzymes involved (red thought bubble)

2.5.3 Physiological effects of ethylene

Almost all the parts of higher plants produce ethylene at varied concentrations. The rate of ethylene production depends upon the tissue type and the stage of development of the plant organ (Grierson, 2012). The plant hormone ethylene is responsible for numerous beneficial and detrimental effects on the postharvest quality of fruit and vegetables. Most of the effects caused by ethylene were noticed even before it was known that it was the cause of the response. Ethylene plays a major role in the ripening of fruits and its production is also induced as a response to different abiotic and biotic stresses such as wounding, chilling injury, flooding, temperature stress, diseases, insect and pest infestation (Imaseki, 1991). Ethylene gas is directly or indirectly

involved in several ripening-related physical, physiological and biochemical changes in the fruits (Anwar et al., 2018).

Ethylene is responsible for different physiological effects in plants from seedling to organ senescence. The age-old expression “One rotten apple spoils the whole bushel” has a scientific basis. Ethylene, released from ripe, diseased or injured fruit, has been recognised and proved as a hormone which accelerates the ripening process in many edible fruits (Burg and Burg, 1962). However, not all fruits respond the same to ethylene exposure. On exposure to external ethylene gas, the climacteric fruits exhibit increased internal ethylene production, accelerate the ripening process and the action could be autocatalytic up to a certain point. The climacteric fruits exhibit a sharp rise in ethylene production along with respiration, prior to the onset of ripening on or off the plant. The size and onset of the climacteric peak may vary among different fruits. Fruits with higher peaks tend to ripen early and deteriorate faster (Abeles et al., 1992). In general, apple fruit are categorised as very high ethylene producing fruit (>100 µL $kg^{-1}h^{-1}$) and highly sensitive to external ethylene exposure. Whilst, the pear fruit are high ethylene producing (10-100 µL $kg^{-1}h^{-1}$) and highly sensitive to external ethylene exposure (Abeles et al., 1992). Commercially, the climacteric fruits such as mango, banana and avocados are harvested at the green mature stage, a physiological stage just before ripening initiation. These fruits are then brought to the ready-to-consume stage by the ethylene treatment in controlled conditions, which induce uniform ripening with consistent appearance and quality (Mahajan et al., 2010a; Blakey et al., 2012). Unlike climacteric fruits, non-climacteric fruits do not exhibit any significant peak of respiratory activity and show a gradual decline during ripening. Exogenous ethylene exposure does not cause any significant changes in ripening activity of non-climacteric fruits but can induce the colour in the pericarp of some fruits such as Citrus group, by the degradation of chlorophyll and development of other colouring pigments (Ortuno Tomás et al., 1993, Tucker, 2012).

In nature, ethylene along with abscisic acid enhances the senescence process in plant organs such as flowers, fruits and leaves. In the majority of plants, ethylene exposure accelerates the ageing process in plant parts, which is characterised by degradation of chlorophyll, proteins and nucleic acids. Fruit ripening and senescence induced by ethylene are oxidative processes and involves the formation of several reactive oxygen

species (ROS), which in turn are responsible for the depletion of bioactive constituents and other antioxidant compounds (Masia, 1998). The oxidative metabolism in plants is ethylene-dependent and is responsible for the production of ROS (Steinite et al., 2004). The postharvest life of horticulture produce is linearly increased with logarithmic decrease in ethylene from 10 to 0.001 μL L^{-1} (Wills et al. 2001, Pranamornkith et al. 2012). Ethylene also possesses a natural role in abscission of plant parts/organs (separation from parent plant) (Arteca, 2013). In addition to the above mentioned, ethylene was also found to be involved in several other plant physiological processes such as seed germination, embryogenesis, bud dormancy, sexual development and others (Arteca, 2013).

2.5.4 Ethylene signal transduction

In fruit, the ethylene gas diffuses freely from one cell to another across the membranes and integrates the ripening processes. The autocatalytic property of ethylene enhances its coordinating function in plants. Ethylene gas is very effective in causing physiological changes in plants even at very low concentrations and hence this suggests the presence of a high-affinity receptor system for ethylene in plants (Abeles et al., 1992). The ethylene action in the plants starts with binding to an appropriate receptor to activate one or more signal transduction pathways. Ultimately, they lead to a cellular response by altering the gene expression pattern (Figure 2.11).

The ethylene perception in plants is well understood through the molecular genetic studies of *Arabidopsis thaliana* (Rodriguez et al., 1999). The amino acid sequences ETR1, ETR2, ERS1, ERS2 and EIN4 are identified as ethylene receptors and any anti-sense mutations in the genes encoding these proteins prevent the binding of ethylene to the receptor and ultimately inhibit the regular ethylene response pathway. All the ethylene receptors possess an ethylene-binding site, which is hydrophobic and readily combines with ethylene. The presence of Copper (I) cofactor is essential to coordinate with ethylene receptor ETR1 and for high-affinity binding with ethylene molecule (Rodriguez et al., 1999). RAN1 protein assembles the Copper (I) cofactor into the ethylene receptor and helps in receptor binding. The ethylene binds to ETR1 protein and on binding, it then activates CTR1, a downstream protein. CTR1 communicates with EIN2, a transmembrane protein to pass the stimulus to the nucleus. The CTR1 protein gets inactivated as soon as it activates the EIN2 protein. The EIN2 protein

transfers stimulus to the nucleus by travelling through the nuclear membrane and further activates EIN3 and ERF1 proteins in the nucleus. These proteins modulate appropriate gene expression for the plant/ plant organ action in response to ethylene exposure (Taiz et al., 2015).

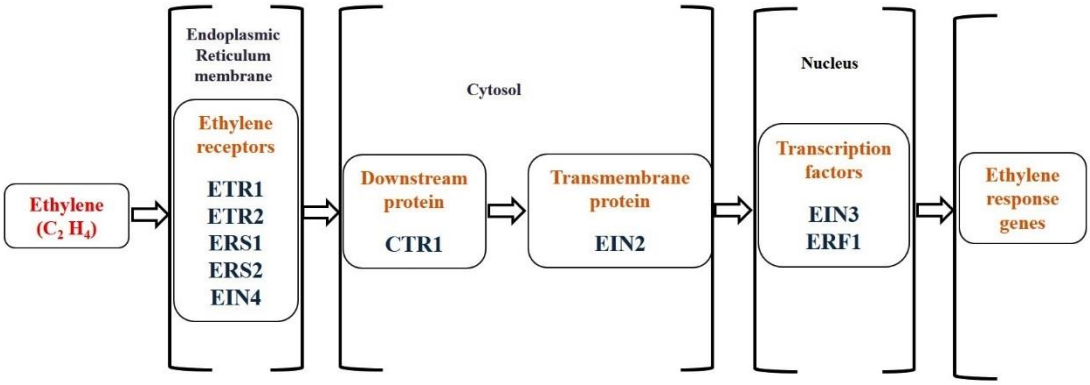


Figure 2.11 Ethylene signal transduction pathway

2.5.5 Ethylene agonists

Agonists are the compounds which bind to the receptors and activate the receptors to produce a biological response. Several alkenes and alkene-related compounds are considered ethylene agonists exhibiting similar responses in the plants as ethylene does. Propylene, acetylene, and carbon monoxide were proved to cause actions in plants very similar to ethylene (Sisler, 1979). Propylene exhibited similar effects as ethylene in banana (McMurchie et al., 1972), apples (Sfakiotakis and Dilley, 1973), kiwifruit (*Actinidia deliciosa* (A. Chev.)) (Sfakiotakis et al., 1989), pears (Gerasopoulos and Richardson, 1996), plum (Abdi et al., 1997) and acetylene in mangoes (Medlicott et al., 1987).

2.5.6 Ethylene regulation

Ethylene accelerates postharvest ripening process and deterioration of horticulture produce. It is estimated that ethylene is responsible for up to 50% postharvest losses in fruits and vegetables (Blanke, 2014). Capacity to store the fruits for long periods will increase prospects for long-distance markets and hence maximise the profits. Techniques to inhibit these undesirable effects of ethylene are very important tools to reduce postharvest losses and enhance storage life. The negative effects of ethylene

can be inhibited at two different stages i.e., by inhibition of biosynthesis and by inhibiting the action.

2.5.6.1 Inhibition of ethylene biosynthesis

Formation and oxidation of ACC are essential steps in ethylene biosynthesis. These steps are catalysed by ACS and ACO enzymes, respectively. There are different compounds available which inhibit the activity of these enzymes and hence inhibit ethylene biosynthesis (Figure 2.12). 1-aminoethoxyvinylglycine (AVG), methoxyvinylglycine (MVG) and amino oxyacetic acid (AOA) inhibit ACS activity, while α-amino isobutyric acid (AIB) and ethanol are some of the compounds which suppress ACO activity (Martínez-Romero et al., 2007). Higher concentrations of carbon dioxide in the storage environment also reduce the autocatalytic production of ethylene in climacteric fruits (Abeles et al., 1992).

Application of AVG at both preharvest and postharvest stages significantly reduced the ethylene production in fruits such as apples, pears, peaches (*Prunus persica* L., Batsch), plums and other fruits (Bregoli et al., 2002; Jobling et al., 2003; Batur and Çetinbaş, 2017; Doerflinger et al., 2019). Postharvest application of ethanol or acetaldehyde vapours has significantly reduced ethylene biosynthesis through inhibition of ACO activity in apples (Pesis, 2005), peaches and nectarines (*Prunus persica* var. nectarine (L.) Batsch) (Lurie and Pesis, 1992). Ethanol application also reduced superficial scald in Granny Smith apple fruit (Scott et al., 1995), but did not show any effect in grapes (Pesis and Marinansky, 1992) and banana (Bagnato et al., 2003). Cobalt (Co^{2+}) application effectively blocked autocatalytic ethylene biosynthesis by inhibiting ACO activity in apples (Tian et al., 1994), but due to its toxicity, its usage is restricted in fruit crops. Nevertheless, they are used to prolong the vase life of flowers by inhibition of ethylene production (Staby et al., 1993).

Unlike the above-mentioned compounds polyamines (PAs) such as spermine (Spm), spermidine (Spd) and putrescine (Put) compete with ethylene for SAM and hence affect ethylene biosynthesis. Both PAs and ethylene have common precursor SAM for their biosynthesis in plants (Valero et al., 2002). Exogenous application of PAs enhances the production of endogenous PAs in plants and control ethylene production during ripening processes (Pandey et al., 2000). PA application reduced ethylene

biosynthesis in apples (Wang et al., 1993), plums (Pérez-Vicente et al., 2002) and other fruits (Sharma et al., 2017).

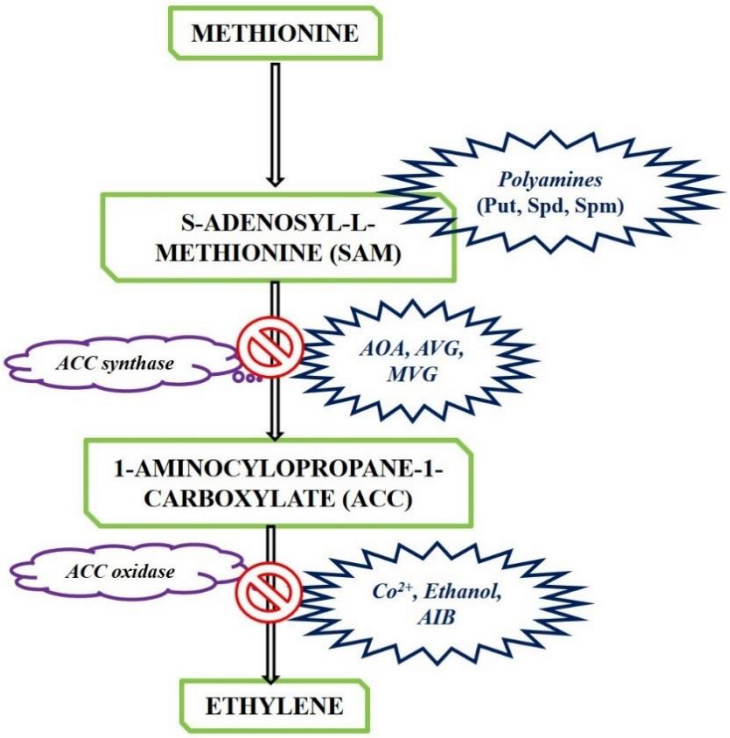


Figure 2.12 Inhibition of ethylene biosynthesis at different stages of biosynthesis pathway

2.5.6.2 Inhibition of ethylene action

Antagonists are the compounds that bind to the receptors and block the respective action. Plant physiological responses due to ethylene can be antagonised using different chemical adjuvants (Sisler et al., 2006). Sisler et al. (2006) reviewed in detail about different ethylene antagonists and their role in blocking ethylene action. Silver thiosulfate is very effective ethylene inhibitor. The silver ions (Ag^{+}) substitute copper ions (Cu^{2+}) at ethylene binding site and interfere with ethylene response. But being a heavy metal, the usage of silver salts is banned in fruit and vegetables due to possible toxicity (Rodriguez et al., 1999).

a. Cycloalkenes

Sisler et al. (2006) have tested several cyclic alkene compounds for their effectiveness in inhibiting ethylene action. Some of the ethylene antagonists require continuous exposure to exhibit effective inhibitory action. 2,5 Norbornadiene (2,5-NBD) efficiently antagonised the ethylene action among all the cyclic alkenes tested. *Trans*-cyclooctene (TCO) (Sisler et al., 1990), cyclopentadiene and cyclobutene were some of the other cycloalkenes, which were found as effective as 2,5-NBD. 2,5-NBD effectively reduced abscission suppressed activity of cell wall hydrolysing enzymes in citrus explants (Sisler et al., 1985). However, its commercial application was restricted due to its pungent and unpleasant odour (Sisler et al., 2006).

b. Terpenes

Grichko et al. (2003) tested different terpenes for the ethylene antagonist activity and reported that several natural terpenes compete with ethylene to combine with the receptors. Isoprene, limonene, *trans*-cinnamaldehyde, eugenol, cinnamyl alcohol, trans-2-hexane-1-ol, α-pinene, α-terpinene, (+) carvone and carveol are some of the plant-origin compounds tested for antagonistic action. It was found that almost all the terpenes showed antagonistic activity, but their effectiveness in antagonising ethylene action varied. Fumigation with limonene effectively antagonised ethylene action in waxflowers (*Chamelaucium* spp.) (Abdalghani et al., 2018).

c. Cyclopropenes

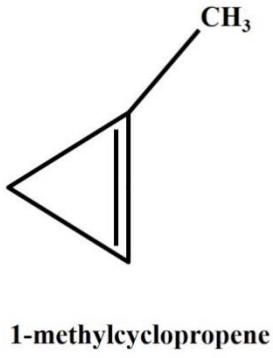
Ring strain in the organic compounds is usually used to measure the instability and reactivity of cyclic compounds (Gordon, 1980). The ring strain of the compound determines its affinity towards the receptors (Levitzki, 1984), however, in the case of ethylene antagonists, the strain values alone cannot be considered as a scale to judge effectiveness. Though cyclopropenes possess high ring strain values, alone it cannot act as ethylene antagonists as it lacks a double bond (Sisler et al., 2006). Cyclopropenes (CP) are effective ethylene inhibitors but are chemically very unstable and need to be stored at very low temperatures (Schipperijn and Smael, 1973). Unlike other cycloalkenes, the antagonistic effect of cyclopropenes persist longer and even a

one-time exposure can induce ethylene inhibition for a significant number of days (Sisler et al., 2006).

Different forms of cyclopropenes such as 1-methylcyclopropene (1-MCP), 3-methylcyclopropene (3-MCP), 3,3-dimethylcyclopropene (3,3-DMCP), 1-ethylcyclopropene (1-ECP) and other possible CP analogues were tested for its efficiency as ethylene action inhibitor and found that all the forms antagonised ethylene action (Sisler et al., 2006; Apelbaum et al., 2008). The capacity of the inhibitor to antagonise ethylene action varied depending upon the molecular size and structure. Even though the empirical formula of 3-MCP and 1-MCP are similar, the effectiveness in antagonising ethylene action and stability of 3-MCP was significantly less than 1-MCP (Sisler et al., 2006). As per the reports of Apelbaum et al., (2008) even the different plant organs respond differently to the same inhibitor, signifying differences in ethylene receptor structures and their availability in various tissues within the same plant.

2.6 1-Methylcyclopropene (1-MCP)

1-MCP is comparatively more stable CP at the gaseous state and effectively inhibit ethylene action for several days. It is CP with hydrogen in 1st position replaced by a methyl group (CH_3) (Figure 2.13). Nevertheless, 1-MCP is unstable at room temperature in the liquid state, with a boiling point near 0°C (Sisler et al., 2006).



1-methylcyclopropene

Figure 2.13 Structure of 1-methylcyclopropene

FloraLife Inc. (Walterboro, South Carolina, USA) developed a γ-cyclodextrin matrix to encapsulate the reactive molecule of 1-MCP and marketed the product

commercially as EthylBloc®. This stable matrix when dissolved in water release 1-MCP in a gaseous form (Sisler, 2006). 1-MCP as EthylBloc® received approval from the Environmental Protection Agency (E.P.A.) in 1999 for commercial usage in the ornamentals. AgroFresh Inc. further developed 1-MCP formulation and received rights to use 1-MCP on edible fruit and vegetables under trade name SmartFresh™ (Watkins, 2006). Recently, AgroFresh Inc. has developed liquid formulations of 1-MCP under trade name Harvista™, specially designed as a preharvest foliar spray to control preharvest fruit drop in apples, but the tests are still in progress for commercialisation (Watkins, 2015).

1-MCP is a non-toxic compound and there were no detectable residues found in the fruit and vegetables treated with it. Following the E.P.A. approval (E.P.A., 2002), 1-MCP has been registered for commercial usage in the horticulture industry in several countries for different fruit and vegetable crops (Watkins, 2006). Since then, several researchers tested 1-MCP with different fruit and vegetables and recorded significant reduction in ethylene production, the rate of respiration, colour change and softening (Blankenship and Dole, 2003; Martínez-Romero et al., 2003; Sisler, 2006; Watkins, 2006; Martínez-Romero et al., 2007; Watkins, 2008; Schotsmans et al., 2009; Watkins, 2015; Li et al., 2016; Valero et al., 2016). The efficacy of the 1-MCP treatments varied with concentrations and duration of treatment, fruit species, cultivars, storage conditions, presence of exogenous ethylene and maturity stage (Schotsmans et al., 2009; Escribano et al., 2017). The effective concentration of 1-MCP again depends upon factors such as fruit species, variety and storage conditions. Sisler et al. (2006) opined that the difference in effective concentrations within different cultivars and fruits could be due to the difference in rates of ethylene receptor synthesis. However, each registered country has established some permitted maximum treatment concentrations for commercial application of 1-MCP. The maximum permitted treatment concentrations for apples is 1 µL L^{-1} (18 µM) (Watkins, 2006). The potency of the antagonists also varied with maturity stage and highest efficiency of any inhibitor was obtained only when the produce was treated before the onset of the ripening process/ethylene action (Apelbaum et al., 2008).

Commercially 1-MCP is being applied in the form of fumigation in the sealed rooms. But with recent development of Harvista™ (liquid 1-MCP formulation) by AgroFresh

Inc., the effects of preharvest spray and postharvest dip treatments of 1-MCP have been evaluated in 'Joanna Red' plums (Manganaris et al., 2008), 'Hass' avocado (Choi et al., 2008), Bartlett' pears 'Empire' (Escribano et al., 2017) and 'McIntosh' apples (Doerflinger et al., 2019). All the authors reported that treatment with liquid 1-MCP reduced rates of ethylene and respiration and delayed fruit softening.

2.6.1 Mode of action of 1-MCP

1-MCP is a powerful ethylene antagonist and inhibits ethylene action through the interaction with the ethylene receptors. It is very active even at low concentrations and out-competes ethylene in interacting with ethylene receptors. Compared to ethylene 1-MCP is 10 times more potent in combining with ethylene receptors (Blankenship and Dole, 2003). It blocks ethylene binding sites of the receptors irreversibly and thereby prevents the expression of ethylene response genes (Watkins, 2006).

Sisler and Serek (1997) proposed ethylene antagonistic nature of 1-MCP and other CPs through ligand substitution model. The model explains that ethylene or 1-MCP binds to metal centre surrounded by ligands of unknown structure. In case of the ethylene, the ligand gets substituted and expels ethylene molecule resulting in expression of ethylene response genes, whilst, the 1-MCP binds too tightly to the metal centre and blocks the formation of the active receptor complex.

Later Pirrung et al. (2008) proposed a cyclopropene ring-opening reaction model. According to this model, 1-MCP binds with copper metal by the double bond. The back bonding from the copper metal initiates CP ring-opening reaction to form a copper carbenoid intermediate. The intermediate formed then reacts irreversibly to form covalent bonds with adjacent amino acids of the protein domain of receptor sites and inactivate/ damage the ethylene receptor. Hence the ethylene action is blocked (Burg and Burg, 1967; Pirrung et al., 2008).

2.6.2 Effects of 1-MCP on postharvest physiology and quality of the fruit

Several authors reviewed in detail about different effects of the 1-MCP, practical concentrations and responses, working mechanism in different climacteric and non-climacteric horticulture crops (Blankenship and Dole, 2003; Martínez-Romero et al.,

2003; Sisler, 2006; Watkins, 2006; Martínez-Romero et al., 2007; Watkins, 2008; Schotsmans et al., 2009; Watkins, 2015; Li et al., 2016; Valero et al., 2016).

1-MCP blocks ethylene signal transduction pathway and inhibits the ethylene action. It also reduced/inhibited ethylene production in several fruits most likely by inhibiting the activity of ACS and ACO involved in the conversion of SAM to ACC and then to ethylene. Downregulation of ACS and ACO activity by 1-MCP was reported in apple fruit (Dal Cin et al., 2006), pear (Villalobos-Acuña et al., 2011), banana (Zhang et al., 2006). But, no marked effect of 1-MCP was observed in the ACS activity of peaches (Mathooko et al., 2001, 2004; Dal Cin et al., 2006). This suggests that 1-MCP is effective only in the conditions/developmental stages where the expression of a certain character is controlled by specific ethylene-regulated genes (Schotsmans et al., 2009).

The rate of respiration reduced significantly in most of the fruits on treatment with 1-MCP. In the case of climacteric fruits, the onset of the climacteric peak was delayed along with a reduction in respiration rates (Valero et al., 2016). Application of 1-MCP significantly reduced the levels of ethylene production as well as delayed or reduced the climacteric ripening-associated sharp rise in internal ethylene concentrations in apple and pear fruits. The extent of the delay or inhibition depends upon the cultivar, storage type and length of the storage (Watkins, 2006). Whereas in the non-climacteric fruit like sweet cherry (*Prunus avium* L.), 1-MCP showed no significant effect on respiration rates (Gong et al., 2002). This indicates the presence of different types of receptors in different fruits and some receptors which cannot bind to 1-MCP.

Treatment with 1-MCP delayed/inhibited colour development in the majority of the fruit by delaying biosynthesis of pigments such as carotenoids and anthocyanins (Watkins, 2006). The fruits treated with 1-MCP exhibited significant delay in chlorophyll degradation process (Wang et al., 2006). It has been reported by several researchers that application of 1-MCP inhibits/delays a colour change in the fruit skin of apple and pear (Watkins, 2006). While Johnston et al. (2009) reported that degreening in apple fruit is ethylene independent and Dauny and Joyce (2002) detected no effect of 1-MCP on the skin colour of apple fruit.

Ethylene is responsible for the initiation of flavour development in the fruits. The production of aroma volatiles remarkably reduced in the 1-MCP treated fruits

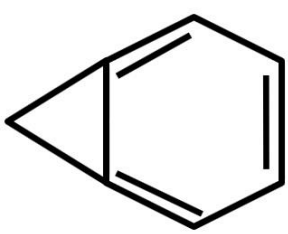
(Schotsmans et al., 2009). In apple fruit, the ester and alcohol production was delayed when treated with 1-MCP, but the levels of aldehydes remained similar to the untreated fruit (Defilippi et al., 2004).

The flesh firmness was retained significantly higher in most of the fruits treated with 1-MCP when compared to untreated. But the extent and duration of this effect varied with fruit species, cultivar, maturity stage and storage conditions (Huber et al., 2003). 1-MCP treatment also reduced the physiological loss of weight (PLW) in apple and pear fruit and maintained the cell turgor during storage at room temperature (Baritelle et al., 2001). Mir et al. (2001) treated 'Redchief Delicious' apple fruit and found that the fruit firmness was retained even when kept at room temperatures (20-24°C). Contrarily, Toivnen and Lu (2005) found that the effect of 1-MCP in retaining fruit firmness on early ripening 'Sunrise Summer' apple was lost at 15°C.

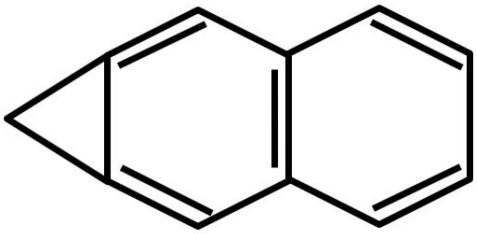
Varied responses have been reported by different authors for the effects of 1-MCP treatments on the nutritional quality of fruits. MacLean et al. (2006) recorded higher levels of antioxidant levels in apples treated with 1-MCP, while Larrigaudière et al. (2005) reported that 1-MCP treated apple fruit showed no significant difference in ascorbic acid and antioxidant levels when compared to untreated fruits.

2.7 Novel ethylene antagonists

Singh et al. (2018) formulated two compounds 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) (Figure 2.14), which are structurally different from 1-MCP but possess the capacity to retard ethylene responses in plants or plant organs. The inventors claimed that these compounds could effectively retard a range of ethylene responses such as respiration rate, fruit softening, colour development, abscission, senescence and ultimately extend the storage life of produce while ensuring the maintenance of commercial fruit quality. Previously, Khan (2014) and Abdalghani (2017) reported the potential of BC and NC as ethylene antagonists in few cultivars of plums, apples and waxflowers. These compounds are yet to commercialised.



1 *H*-cyclopropabenzene



1 *H*-cyclopropa[*b*]naphthalene

Figure 2.14 Structure of 1 *H*-cyclopropabenzene and 1 *H*-cyclopropa[*b*]naphthalene

2.7.1 Proposed mode of action

The mode of action of BC and NC is similar to that of 1-MCP as explained in Section 2.6.1 (Musa, 2016). These compounds react with copper (I) cofactor situated within the ETR1 ethylene receptor and cleave cyclopropene ring forming a copper carbenoid intermediate. The intermediate complex then reacts with the nucleophile adjacent to the binding site forming a covalent bond. The antagonistic effect is then caused by blocking the receptor from interacting with the ethylene molecule and ultimately inhibiting expression of ethylene response genes in the plant cell.

2.8 Storage environments

Management of storage environment is a crucial factor in extending postharvest storage life and to delay the quality deterioration of the fruits (Domínguez et al., 2016). Maintaining low temperatures and high humidity is essential factor to preserve quality and safety of fresh fruit during storage as well as throughout supply chain (Gross et al., 2016). Cold storage controlled atmospheric (CA) storage, modified atmosphere packing (MAP) are common methods used for long-term storage of fruits. There are several reports of synergistic effects of combined application of ethylene antagonists and cold storage or CA storage, on the storage life and fruit quality of apple and pear fruits.

2.8.1 Cold storage

Cold storage or low-temperature storage proved to be a reliable source to extend the storage life of several horticulture crops (Kitinoja, 2013). It is one of the oldest storage

methods being practised by humans for long-duration storage of food commodities. Fruits are the living tissues which respire and undergo different physiological changes even after separation from the parent plant. Presence of higher temperatures in any step of the supply chain accelerates natural degradation processes in food commodities resulting in loss of natural colour, flavour, texture and nutrient levels. According to 'Q_{10} temperature coefficient' principle the rate of degradation enzyme activity doubles for every 10°C rise in temperature. It can be related that the storage life of produce can be doubled by every 10°C reduction in storage temperature (Kitinoja, 2013). Storing the fruits at low temperatures elongate the storage life of fruits by reducing the rate of respiration, lowering ethylene biosynthesis rate and decreasing water loss (Kitinoja and Kader, 2002; Keller et al., 2013; Gross et al., 2016). Low-temperature storage has also maintained fruit firmness, nutritional attributes and organoleptic properties of most of the fruits (Paull, 1999). Some temperate fruits like pears require exposure to chilling temperature for a certain period to initiate the normal ripening process (Bai et al., 2009). The duration of chilling requirement in pear fruit varies with the cultivar like 2-4 weeks for Bartlett pear (Agar et al., 1999), 2-3 weeks for Bosc pear (Chen et al., 1982) and 7-8 weeks for d Anjou pear (Chen et al., 1982).

The response to cold temperatures varies with different fruit species, varieties, preharvest practices, postharvest handling before storage and length of the storage. In general, the temperate fruits such as apples, cherries and pears could be stored up to 0°C safely without any negative effects. But, in case of the tropical and subtropical fruits such as banana, mango and sweet orange when stored at very low temperatures (less than 7-10 °C), they develop chilling injury symptoms. These symptoms include black blemishes (sweet orange, mangoes and bananas), brown discolouration (avocados), decay (cucumber, leafy vegetables) and development of off-flavours (tomato). The list of safe temperatures and storage conditions for different fruits and vegetables are well documented by Gross et al. (2016).

The recommended lowest temperatures for storage of apple fruit depends on the cultivar and in general, it must be above freezing temperature (0°C) to avoid chilling injury and below 4°C to achieve advantages of cold storage. The rate of respiration is significantly lowered in the apple fruit during low-temperature storage (Watkins, 2003). The rates of respiration of apple cultivars susceptible to chilling injury may be

stimulated by cold storage. 'Idared' apple fruit stored at 0°C exhibited lower respiration rates initially when compared to the fruit stored at 2°C and 4°C, but later with the extension of the storage period, the rates of respiration were higher (Johnson and Ertan, 1983). Similarly, the rise or fall of ethylene production and the onset of ethylene peak in the apple and pear fruit stored in cold storage is strictly cultivar specific (Knee et al., 1983).

2.8.2 Controlled atmospheric (CA) storage

CA storage works on the principle of manipulation of the gas concentrations in a storage room, especially levels of carbon dioxide (CO_2) and oxygen (O_2). It involves elevated levels of CO_2 ≥1% and reduced O_2 levels ≤ 8% in the storage chambers. The modifications in the atmosphere of CA storage is considered only as supplementary and the lowest safe temperatures and high relative humidity (90 – 95 %) are still to be maintained to preserve the nutritional status and other quality parameters of the fruit (Gross et al., 2016).

CA effectively extends the storage life of fruits by lowering rates of respiration, ethylene biosynthesis, senescence and other ripening-related physiological changes (Keller et al., 2013). The autocatalytic production of ethylene in climacteric fruits is inhibited at higher CO_2 levels (Abeles et al., 1992). The gas concentrations in CA are unfavourable for insect pests and growth of some fungi and hence help in reducing damage due to pest and diseases during storage (Kader and Rolle, 2004; Saltveit, 2003).

The optimum concentrations of O_2 and CO_2 in CA storage varies with fruit crop, species, cultivar, storage temperatures, pre-harvest conditions and stage of maturity at harvest. The stress caused by too low concentrations of O_2 can aggravate physiological disorders such as internal browning in apple and pear fruits. Very high CO_2 levels create anaerobic conditions and develop off flavours and odours in the fruit. (Thompson, 2010; Gross et al., 2016). Thompson (2010) has discussed in detail the threshold concentration of O_2 and CO_2 gases in the CA storage to store different cultivars of apple and other horticulture produce along with a list of symptoms. In general, the CA storage recommendations for apples include 1–2 % CO_2 + 2–3 % O_2 for non-chilling-sensitive cultivars and 2–3 % CO_2 +2–3 % O_2 for chilling-sensitive

cultivars (Gross et al., 2016). The recommended CA storage environment for pear fruit range between 0–2.5 % CO_2 + 0.5–3 % O_2 (Sugar, 2007).

The storing 1-MCP treated apples and pears in CA storage enables long-term storage of fruit while maintaining consumer acceptable fruit quality. The fruit quality was effectively maintained in the 1-MCP treated fruit stored in CA when compared to untreated fruit stored in CA. The different apple cultivars treated with 1-MCP maintained comparatively higher fruit firmness, titratable acidity and phenols when stored in CA storage (Watkin et al., 2000; DeLong et al., 2004; DeEll et al., 2005; Bai et al 2005). The effectiveness of the combined application of CA and 1-MCP depends upon the cultivar used, stage of maturity at harvest, and storage temperatures (Bai et al., 2009). The pome fruits stored in CA tend to show a sharp rise in ethylene production after being removed from CA or cold storage. The fruit treated with ethylene antagonist retards the ethylene production after removing from storage and allow longer shelf-life in the markets (Watkins, 2006; Mattheis, 2008; Bai et al., 2009).

2.9 Ozone

Ozone is named after the Greek word '*ozein*', which means smell. In 1839, it was first discovered by C.F. Schönbein, a German Chemistry Professor as a gaseous substance with a characteristic smell and formed by electrolysis of oxygen (Rubin, 2001). Ozone is made of three oxygen atoms arranged in an obtuse isosceles angle at a bond angle of 116° 49' (116.8°) and bond length of 1.278 Å (Figure 2.15). The melting point and boiling point of ozone are as low as -251 °C and -112 °C, respectively (Beltran, 2003). In 1866, Jacques-Louis Soret determined the molecular formula of ozone and then Bailey (1978) defined the molecular structure of the ozone as a resonance hybrid of the four canonical forms. The ozone molecule actively decomposes to form oxygen and the rate of decomposition is higher as the temperature increases and is relatively stable at low temperatures (Sease, 1976; Xu, 1999). The half-life of the ozone gas is 40 minutes at 20°C, while it can go up to 3 months at very low temperatures of -50°C (Bocci 2011). Ozone is moderately soluble in water and the rate of solubilisation is

The part of the review on 'Ozone' has been included in the book chapter: **Vijay Yadav Tokala**, Zora Singh and Alan D. Payne. 2018. Postharvest uses of ozone application in fresh horticultural produce. In: *Postharvest Biology and Nanotechnology* (Eds. G. Paliyath, J. Subramanian, L-T Lim, K.S. Subramanian, A. Handa and A. Matoo). John Wiley and Sons, Inc. pp. 129-170.

affected by temperature, and the presence of impurities in water (Hill and Rice 1982; Khadre et al. 2001).

Ozone is a strong oxidising agent, with oxidising power more than chlorine or hypochlorous acid. Ozone readily dissociates into superoxide, hydroxyl and hydroperoxyl radicals, which can effectively oxidise most of the organic and inorganic compounds (Manousaridis et al., 2005). In the early 20th century, de La and des Roseaux (1904) explored the germicidal properties of ozone and stated that even dilute concentrations of ozone possess the capacity to disinfect polluted water. Since then, ozone was widely used to purify drinking water, alcoholic beverages, milk and meat products (Hill and Rice 1982).

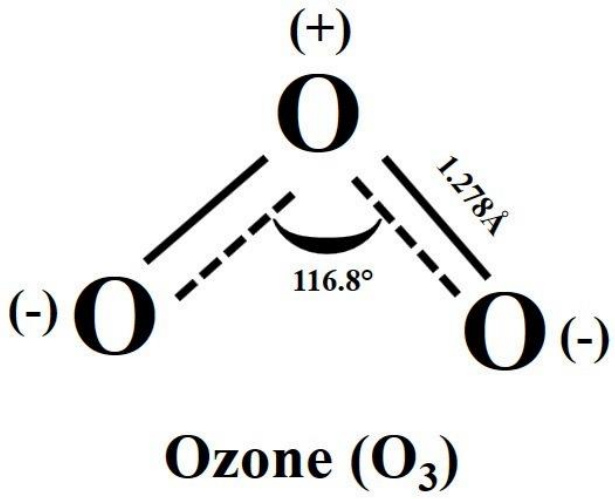


Figure 2.15 Structure of Ozone (O_3) molecule

Pertaining to capacity of ozone to quickly dissociate into breathable oxygen without leaving any chemical residues, it was granted Generally Recognised as Safe (GRAS) status in 1997, approval by the USFDA (United States Food and Drug Administration) and is also permitted for organic certification in the food industry (Selma et al., 2008; Tzortzakis, 2016). The capacity of ozone to reduce or eliminate microbial load on horticulture produce and effects on its physiological and qualitative parameters was evaluated by several researchers and well documented in various reviews (Horvitz and Cantalejo 2014; Glowacz et al. 2015a; Tzortzakis 2016; Tzortzakis and Chrysargyris 2017; Brodowska et al., 2018; Öztekin, 2018; Tokala et al., 2018).

The ozone application acquired a considerable amount of popularity in the horticulture industry due to its flexibility for application in gaseous or aqueous state and for its non-residual nature. Ozone is well-known to effectively decrease the microbial population on the fruit surface and hence reduce losses due to disease incidence (Nadas et al., 2003). Additionally, Ong et al. (1996) reported that ozonated water was effective in reducing the pesticidal residues on the fruit rather than only the tap water.

Application of ozone in aqueous form is more effective than its gaseous form to disinfect the fruit surface. However, the penetration capacity and halftime of ozone in the aqueous state are far less than gaseous. It is also difficult to achieve a higher concentration of ozone in an aqueous state. Gaseous application of ozone in sealed rooms is a more convenient way to treat large loads of fruits at one go (Palou et al., 2001).

Upon extended treatment with ozone, the ethylene molecule is oxidised into carbon dioxide, carbon monoxide and water (Bailey, 1978). Ozone application delayed senescence process and caused significant drop in respiration and transpiration rates in 'Wenzhou' mandarin (*Citrus unshiu* Marc.) fruit by affecting the stomatal opening mechanism (Li et al., 1989). The apple and pear fruits, when treated with ozone in the cold storage, exhibited reduced ethylene levels (Skog and Chu, 2001). Similarly, Zhang et al. (2011) recorded significantly reduced respiration rates in ozone-treated cold-stored strawberry fruit when compared to the untreated. Palou et al. (2001) stated that ozone oxidises ethylene in the storage rooms. They also suggested that it is an efficient way to use ozone generators in the refrigerated containers to allow both ethylene producing (e.g. apples, pears etc.) and ethylene sensitive (e.g. broccoli (*Brassica oleracea* var. *italica*) etc.) as mixed produce during export. Using proteomic studies Minas et al. (2012) has confirmed that ozone impedes ethylene production and ripening process in kiwifruits. However, Liew and Prange (1994) reported that higher concentrations of ozone induce stress and then enhanced the rate of respiration and ethylene production. Palou et al. (2002) observed no significant influence of ozone application on the rate of respiration and ethylene production in peaches and table grapes fruits. The enhanced relative storage life by the application of the ozone was reported in apples (Yaseen et al. 2015), grapes (Sarig et al. 1996), oranges (Palou et al. 2001), kiwifruit (Minas et al. 2010).

Ozone application exhibits higher effectiveness in low-temperature storage environments due to enhanced concentrations of residual ozone (Liew and Prange, 1994). A synergistic effect was observed between cold storage environment and ozone application in delaying ethylene-related physiological changes without any phytotoxic symptoms (Palou et al., 2001, 2002). Levels of total phenolics, total flavanols, and anthocyanins were maintained high in the cold-stored table grapes (Artes-Hernandez et al., 2003, 2007) and kiwifruits (Minas et al., 2010, 2012) treated with ozone. Application of gaseous ozone maintained higher ascorbic acid levels in cold-stored strawberries (Pérez et al., 1999). Contrarily, Zhang et al. (2011) recorded reduced levels of ascorbic acid and antioxidant activity. Combination of ozone and cold storage effectively increased the storage life of fresh fruits while keeping optimum fruit quality (Skog and Chu 2001; Zhang et al. 2011).

Song et al. (2003) recorded a brief rise in the rates of respiration and ethylene production with higher levels of total phenols, anthocyanins and antioxidant capacity in the high blueberries fruits when treated with ozone and stored in CA storage.

Differences in the responses of various fruits to the ozone application could be ascribed to differences in the fruit physiology and ozone concentrations. Irrespective of storage conditions the application of ozone has improved levels of reducing sugars such as fructose and glucose in the fruits. Higher concentrations of ozone effectively reduced the microbial load on the fruit surface but caused a stress-induced rise in respiration rates and phytotoxic symptoms in some fruits. Application of ozone enhanced the levels of antioxidant activity, anthocyanins and total phenols but reduced ascorbic acid and organic acid levels in the fruit (Tokala et al., 2018).

Application of gaseous ozone has retained fruit firmness, reduced physiological loss of weight (PLW), increased shelf-life and reduced spoilage without any phytotoxic symptoms in apple fruit stored in cold storage (Bazarova, 1982; Skog and Chu, 2001; Yaseen et al., 2015). Ozone application in combination with cold (Liew and Prange, 1994) or CA storage conditions (Concha-Meyer et al., 2015) enhanced the beneficial effects in maintaining fruit quality and extending storage life when compared to separate treatments.

2.10 AiroFresh®

The AiroFresh® unit (AiroFresh 1000 AOP) is a new technology by Creative Research Technology (CRT), South Australia, Australia, which claims to be a powerful sterilisation and oxidation technology. It has no filters but utilises the advanced oxidation processes (AOP) and photocatalytic oxidation (PCO) to completely oxidise and degrade airborne contaminants and volatile organic compounds. The PCO can be described as an oxidation reaction that occurs in the photon activated semiconductor (photocatalyst) (Pathak et al., 2017). AiroFresh® functions as an effective purification system with the ability to eliminate gaseous organic compounds including ethylene, aldehydes and exhaust gases. It is also believed to oxidise the fungal spores, inactive bacteria and viruses to destroy them completely. The technology claims to enable a perfect environment in the commercial fruit storage rooms and also induce suitable conditions which allow the maximum efficiency of the ethylene inhibitors (http://airofresh.com.au/) (AiroFresh®, 2019).

Ethylene is believed to be responsible for a major portion of postharvest losses in fruits and vegetables. Regulation of ethylene is an essential tool to reduce these postharvest losses and elongate storage life. There are several techniques applied to inhibit ethylene action but 1-MCP has proved to be the most efficient of all. But 1-MCP is very unstable even at low temperatures and immediately diffuse to gas. It is difficult to use on an industrial scale and not easily available to the growers. 1-MCP is usually available as a service rather than as a chemical and is extremely expensive. There exists a need for alternative non-volatile cyclopropene compounds which are more flexible to use as different possible formulations (Sisler, 2006). This gives scope to explore possibilities of novel ethylene antagonists developed by Singh et al. (2018) as an alternative to 1-MCP. It is also essential to evaluate its effect under different possible storage conditions such as cold storage, CA storage, ozone and AiroFresh®, which are being used commercially.

CHAPTER 3

General materials and methods

3.1 Fruit material

The apple and pear fruit for the different experiments during 2017 and 2018 were obtained from commercial orchards located at various locations in Western Australia (Table 3.1, Figure 3.1). Granny Smith and Cripps Pink varieties are very popular apple cultivars in WA, while Gold Rush pear variety is gaining popularity in recent times. The experiment fruit collected at commercial harvest, were treated with postharvest fungicide dips (described in detail in the respective chapters) and air-dried before packing. All the fruits were packed in the plastic crates/ corrugated cardboard boxes, placing fruits in soft board trays to prevent any mechanical injuries during transit and storage. The fruit were transported from the packhouse to Curtin Horticulture Research Laboratory, Bentley, Perth in an air-conditioned vehicle. The uniformly sized fruit, free from any diseases and bruises were used in different experiments.

Table 3.1 Source of experiment fruit and the locations of orchards

Year	Fruit	Cultivar	Source	Location
2017	Apples	i. Granny Smith ii. Cripps Pink	Newton Brothers Orchards	**Manjimup** 34°13’ S latitude, 116°08’ E longitude
2018	Apples	i. Cripps Pink	Eastwind Farms	**Balingup** 33°45’ S latitude 115°58’ E longitude
	Pears	i. Gold Rush	Casuarina Valley Orchards	**Beedelup** 34°19' S latitude 116°00' E longitude

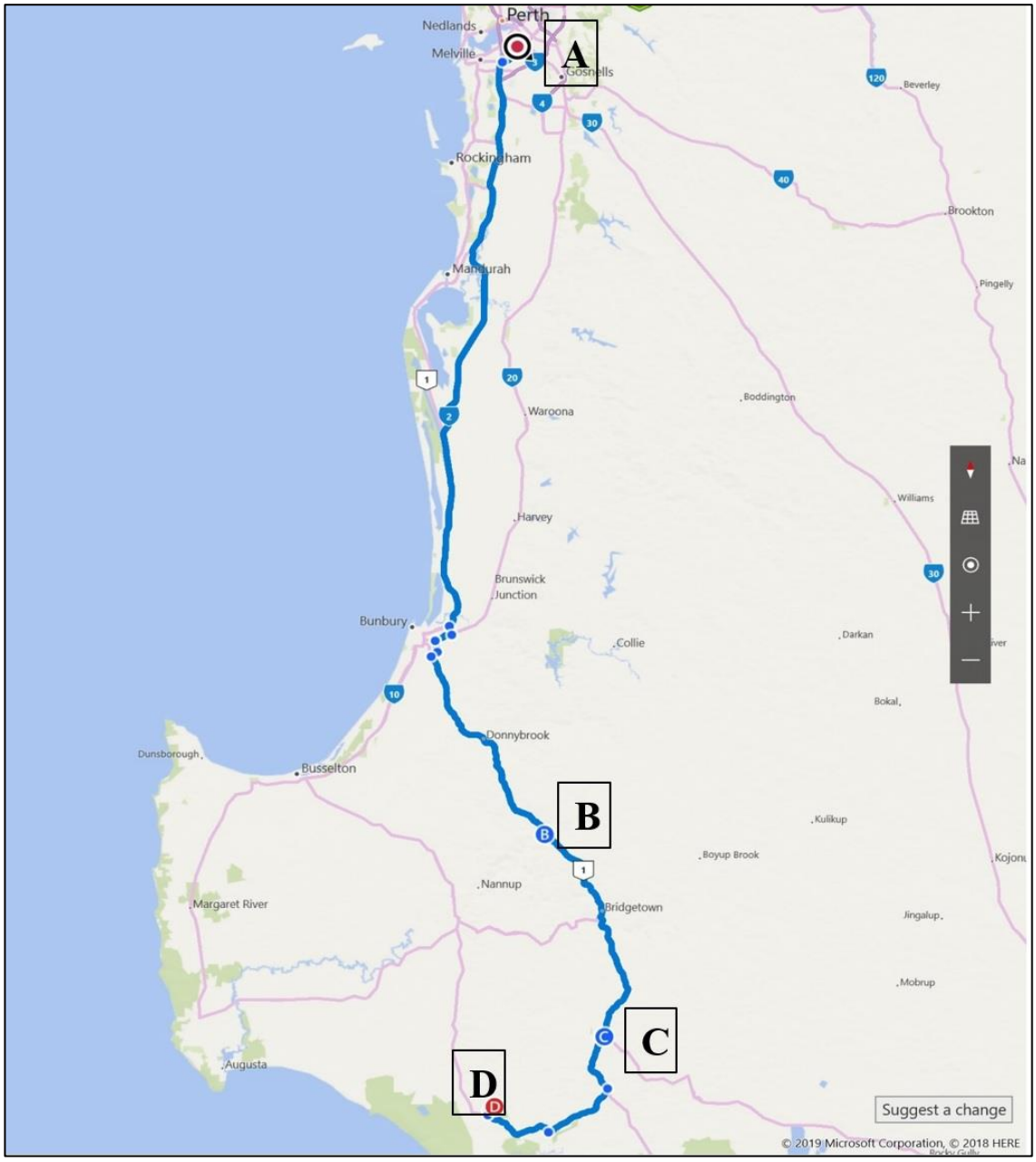


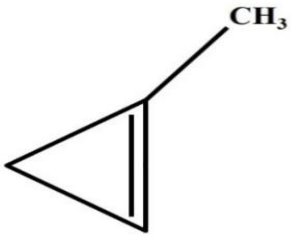
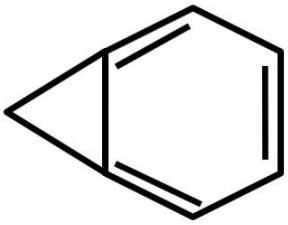
Figure 3.1 Location of orchards in Western Australia, where the experimental fruit was sourced from (B) Eastwind Farms, Balingup (C) Newton Brothers Orchards, Manjimup (D) Casuarina Valley Orchards, Beedelup and transported to (A) Curtin Horticulture Research Laboratory, Perth.

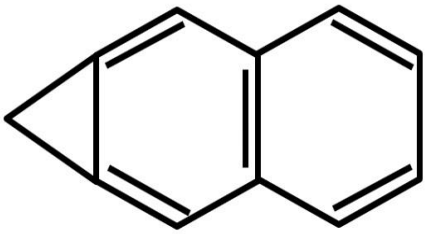
Image source: HERE Maps, Microsoft Corporation. Accessed 15 January 2019.

3.2 Ethylene antagonists

The ethylene antagonists used as treatments in all the experiments were synthesised at chemistry laboratory, Curtin University by Dr Alan Payne, Jason Wells and Jan Sozynshi. Two novel ethylene antagonists (1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) prepared and purified using the procedure described by Davalian et al. (1980) for BC and Billups and Chow (1973) for NC (Table 3.2). The synthesised chemicals were stored in the refrigerator at -80 °C in the glass vials. 1-MCP is the commercially used ethylene antagonist to extend storage life in a range of ornamentals, fruits and vegetables. The 1-MCP was prepared using the procedure mentioned by Fisher and Applequist (1965). 1-MCP was stored as an ethanol solution in the glass vials kept in -80 °C refrigerator. Using the pure form of 1-MCP, instead of the commercial formulation, allowed to compare new ethylene antagonists with 1-MCP preventing any commercial variability. Details of all other chemicals used for qualitative analysis are described in Annexure 1.

Table 3.2 Details of the ethylene antagonists used in different experiments

Ethylene Antagonist	Structure	Chemical Formula	Molecular Weight (g mol^{-1})
1-MCP	 1-methylcyclopropene	C_4H_6	54.09
BC	 1 *H*-cyclopropabenzene	C_7H_6	90.12

NC		$C_{11}H_8$	140.20

1 *H*-cyclopropa[*b*]naphthalene

3.3 Postharvest treatments with ethylene antagonists

3.3.1 Ethylene antagonist fumigation treatment

The experimental fruit were fumigated with the chosen ethylene antagonist in a 60 L hermetically sealable plastic drum. The fruit were carefully arranged on a plastic/metal mesh in the drum followed by fumigation treatments of 1 µM BC (0.090 ppm), 1 µM NC (0.140 ppm), or 18 µM 1-MCP (1.0 ppm or 1 µL L^{-1}). The concentrations of BC and NC used in the experiment were based upon the previous experiments within the research group (Khan, 2014). The calculated amount of ethylene antagonist solution (dissolved in ethanol) be fumigated was poured on to a filter paper placed in a Petri-plate and the drum was immediately sealed air-tight. A small battery-operated portable fan was placed in the drum before sealing it, in order to ensure uniform distribution of the chemical vapour. A Petri-plate with 30 g of granular soda lime was also placed in the drum before sealing it, to absorb excess carbon dioxide (CO_2) produced by the fruit during the respiration process. The control fruit were also sealed in the 60 L drum with soda-lime and portable fan but without any chemicals. The treatments were applied for 18 h at 20 ± 2 °C and 65 ± 5 % RH. On completion of 18 h fumigation period, the drums were unsealed in open-air and the fruit were kept on the soft board trays in the corrugated cardboard boxes to transfer into respective storage rooms according to the experimental conditions.

3.3.2 Ethylene antagonist dip treatment

The experimental fruit were dipped in the 20 L aqueous solutions containing 5 % ethanol alone or ethylene antagonists i.e., 2 µM BC aqueous solution containing 5 % ethanol or 2 µM NC aqueous solution containing 5 % ethanol for 5 min (at 20±2

°C). Maintaining the constant concentration of pure 1-MCP solution at room temperature is difficult as it is highly volatile in the liquid state. So only solutions of BC and NC were prepared for treatment. Following the dip treatments, the fruit were air-dried at open area at room temperature (20±2 °C) with 65±5 % RH before transferring into cold storage, till there are no water droplets left on the fruit surface. The air-dried fruit were arranged on soft board trays in the corrugated boxes and transferred into cold storage according to the experimental conditions. More details included in the relevant experiments.

3.4 Storage conditions

The corrugated cardboard boxes with treated fruits were then transferred to a controlled atmosphere (CA) storage (specific gas combination, temp and RH) or cold storage according to the experimental layout unless otherwise specified. The cold storage (0±2 °C, 90±5 % RH) were equipped with/without ozone generators to produce ozone (O_3) gas with concentration ranging from (0.1 ± 0.08 µL L^{-1}). The CA storage and cold storage were fitted with 'AiroFresh®', which is a unique air purification unit claims to destroy ethylene, mould and bacterial spores, exhaust gases and enable ethylene inhibitors to last for a longer duration with maximum efficiency (AiroFresh®, 2019). The storage period and environments of the individual experiments were based upon the storage potential of the cultivar and the availability of the on-site storage rooms at the fruit growers.

3.4.1 Controlled atmosphere (CA) storage

The experiment fruit were stored in the CA storages at the growers' property maintained at high CO_2 and low O_2 gas concentrations (location details and the exact gas concentrations were described in the respective chapters). The gas concentrations were regularly monitored by the help of infrared gas analyser with automatic sampling.

3.4.2 Cold storage

The cold storage facility at the Curtin Horticulture Research Laboratory or at the growers' property were used during the experiments unless otherwise mentioned for individual experiments. All the cold store was maintained at 0±2 °C, 90±5 % RH. The temperature and relative humidity data during the cold storage period of the

experiment were recorded using Tinytag *Plus* Gemini Data Loggers (Gemini Data Loggers Limited, Sussex, UK) at 15 min interval. The data recorded was probed using the Tinytag Explorer software version 4.7 (Gemini Data Loggers Limited, Sussex, UK).

Figure 3.2 Tinytag *Plus* Gemini Data Loggers used to record temperature and relative humidity data in storage rooms

3.4.3 Ozone

The cold storage (0±2 °C, 90±5 % RH) at the grower's property was installed with ozone generators (Figure 3.3) to produce the ozone gas. The production of ozone gas by these generators involves the passage of oxygen gas through ultraviolet (UV) lamp/ electric field, which initiates dissociation of the oxygen (O_2) molecules into oxygen radicals ($O^{\bullet}$) by the high energy. These radicals collide with other oxygen molecules and result in the formation of ozone molecules. The ozone generators continuously produced ozone gas (0.1 ± 0.08 µL L^{-1}) and the ozone gas concentration was monitored using 'Aeroqual™' series 500 monitor fitted with Aeroqual IP41 Remote Sensor Kit (Aeroqual Ltd., Auckland, New Zealand) (Figure 3.4A, B). The sensor has the capacity to detect the ozone gas concentration ranging from 0 to 10 µL L^{-1} with a minimum detection limit of 0.01 µL L^{-1} and a response time of < 60 s.

Figure 3.3 Ozone generators fitted in the cold store

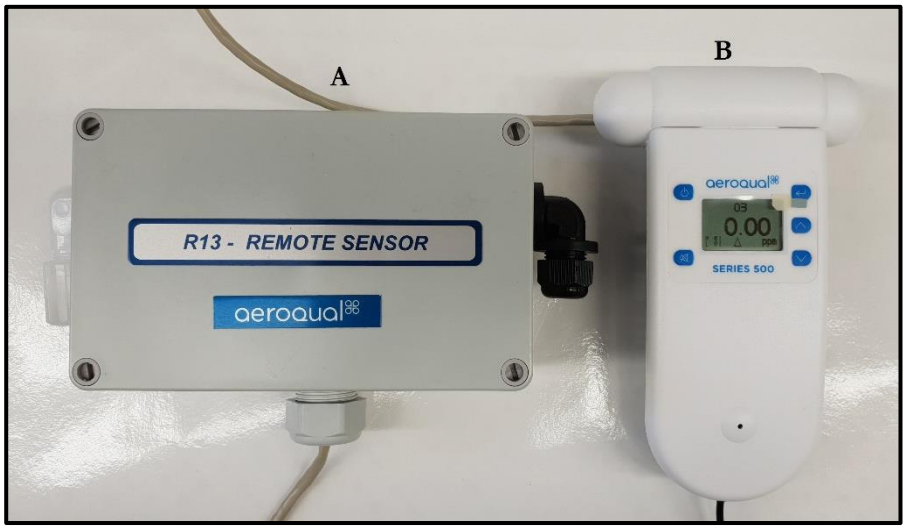


Figure 3.4 (A) Aeroqual IP41 Remote Sensor (B) Aeroqual series 500 monitor

3.4.4 AiroFresh®

The AiroFresh® unit (AiroFresh1000 AOP) by Creative Research Technology (CRT), South Australia, Australia is a new technology, which utilises the advanced oxidation processes (AOP) and photocatalytic oxidation (PCO) to completely oxidise and degrade volatile organic contaminants. It acts as a powerful purification system with the ability to eliminate ethylene, exhaust gases, fungal spores, inactive bacteria and viruses by oxidisation. The technology claims to enable an ideal storage environment in which the ethylene inhibitors can perform to their maximum efficiency (http://airofreshintl.com/) (AiroFresh®, 2019). The AiroFresh® units were installed in the CA storage (Figure 3.5) at the grower's property.

Figure 3.5 AiroFresh® unit installed in the storage

3.5 Determination of fruit physiological parameters

Two fruit per experimental unit free from bruises were randomly selected to determine rates of ethylene production and respiration using the procedure detailed by Zaharah (2011).

3.5.1 Ethylene (C_2H_4) production rate

Two fruit per experiment unit were enclosed in a 1 L glass jar (Fowlers Vacola Australia Pvt. Ltd., VIC, Australia) hermetically sealed by the rubber preserving rings and the lids (Fowlers Vacola Australia Pvt. Ltd., VIC, Australia) (Figure 3.6A). The lid was fitted with an airtight rubber septum (SubaSeal®, Sigma-Aldrich, USA) enabling to draw a gas sample from the headspace of the sealed glass jar. The fruit were sealed in the airtight glass jar for one hour at room temperature (20±2 °C). A one millilitre volume of headspace gas sample from the sealed glass jar containing fruit was then injected into the gas chromatograph (GC) (6890N Network GC system; Agilent Technology, CA, USA) (Figure 3.6B) with 2 m long, 3.18 mm internal diameter stainless steel column and 80/100 mesh size (Porapak-Q, Supelco, PA, USA) and a flame ionisation detector (FID). The nitrogen (N_2) gas was used as carrier gas with 20 mL min^{-1} flow rate. The temperatures of the column, inlet and detector were maintained at 110 °C, 150 °C and 250 °C, respectively. The ethylene in the injected gas sample was identified by comparing the retention time (0.81-0.90 min) with its authentic ethylene gas standard (1.15 ± 0.06 µL L^{-1} of ethylene in nitrogen; BOC Gases Australia Limited, Sydney, Australia). The amount of ethylene production was estimated on the basis of peak areas (Figure 3.7 A, B). In order to check the possibility of the rubber septum emitting ethylene as mentioned by Jacobsen and McGlasson (1970), a procedural blank was run. A gas sample from empty sealed glass jars was injected and no ethylene was detected.

The ethylene production rate (µL C_2H_4 kg^{-1} h^{-1}) was calculated by the following formula and then converted into µmol C_2H_4 kg^{-1} h^{-1} using Ideal gas law (PV = nRT), where

P = atmospheric pressure (atm)
V = volume of the gas (L)
n = number of moles of gas
R = universal gas constant (0.083 L atm K^{-1} mol^{-1})
T = temperature (Kelvin)

$$C_2H_4 \text{ Production } (\mu L \text{ } kg^{-1} \text{ } h^{-1}) = \frac{\text{Conc. of } C_2H_4 \, (\mu L \text{ } L^{-1}) \times \text{Vol. of headspace (L)}}{\text{Fruit weight (kg)} \times \text{Time of incubation (h)}}$$

(A) **(B)**

Figure 3.6 (A) Two fruits sealed in a 1 L airtight jar with a rubber septum (B) '6890N Network Gas Chromatograph system, Agilent Technology' used to estimate the rate of ethylene production

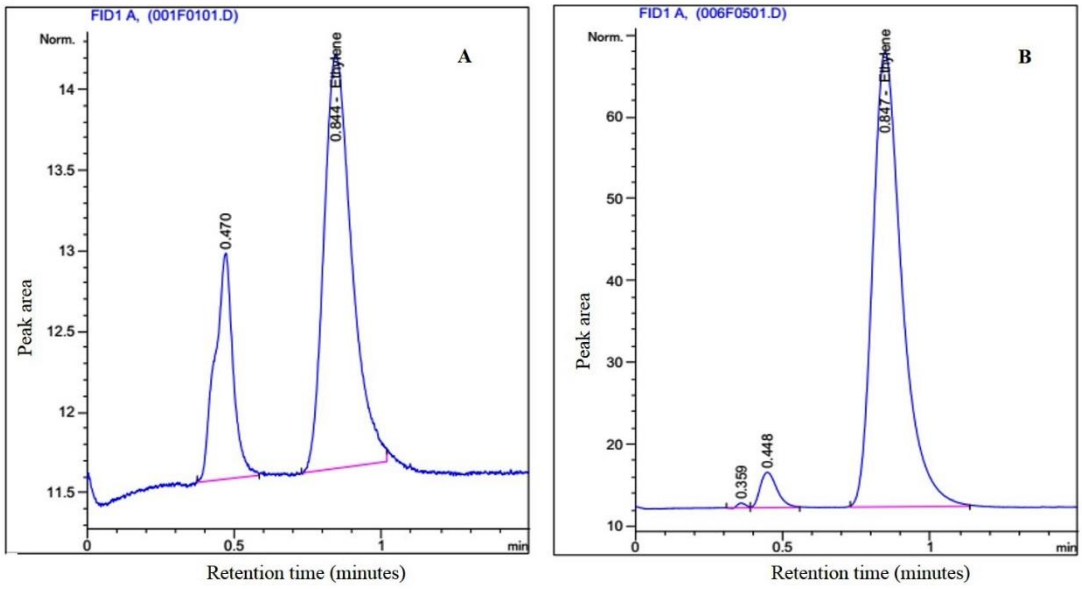


Figure 3.7 Gas chromatographic profiles of ethylene gas production (A) 1.15 ± 0.06 µL L^{-1} ethylene gas standard and (B) gas sample of Granny Smith apple fruit

3.5.2 Respiration rate

The rate of respiration of the fruit sample was estimated based on the amount of carbon dioxide (CO_2) produced during ripening. The two-millilitre headspace gas sample was drawn from the same glass jar (Figure 3.5 A) used for estimation of the rate of ethylene production as explained in Section 3.5.1 and injected into the Infrared gas analyser (Servomex Gas Analyser, 1450 Food Package Analyser, Servomex Limited, UK) (Figure 3.8). The respiration rates were calculated on the basis of peak areas of 2mL standard CO_2 gas (8.31±0.17% CO_2 in N_2 gas, BOC Gases, Perth, Australia) (Figure 3.9). The respiration rate in terms of CO_2 was calculated as mL CO_2 kg^{-1} h^{-1} using the following formula and then converted into mmol CO_2 kg^{-1} h^{-1} using Ideal gas law (PV = nRT).

$$CO_2 \text{ Production (mL kg}^{-1}\text{ h}^{-1}) = \frac{\text{Changes in } CO_2\,(\%) \times \text{Head space volume(L)}}{\text{Fruit weight (kg)} \times \text{Time of incubation (h)}}$$

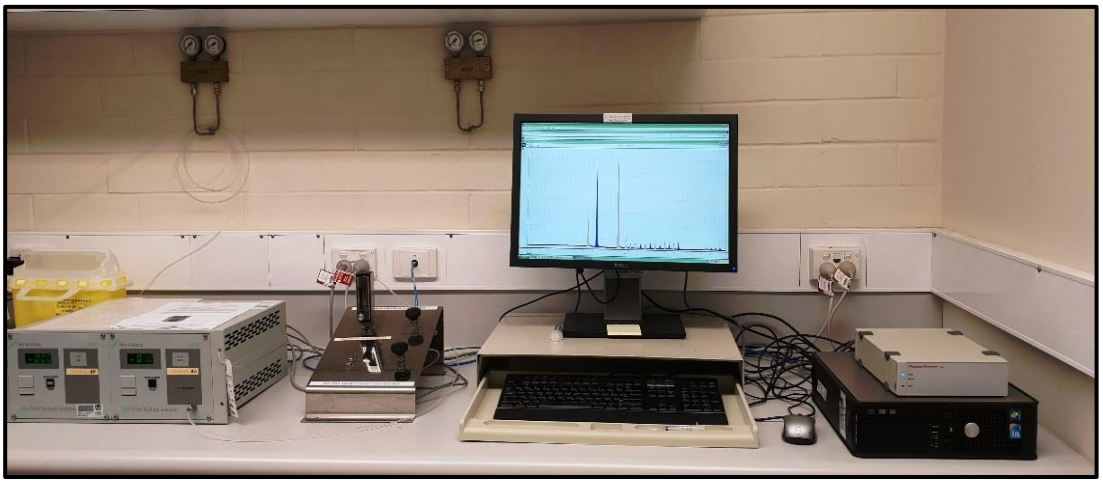


Figure 3.8 ‘Servomex Gas Analyser, 1450 Food Package Analyser’ used to estimate the amount of carbon dioxide evolved

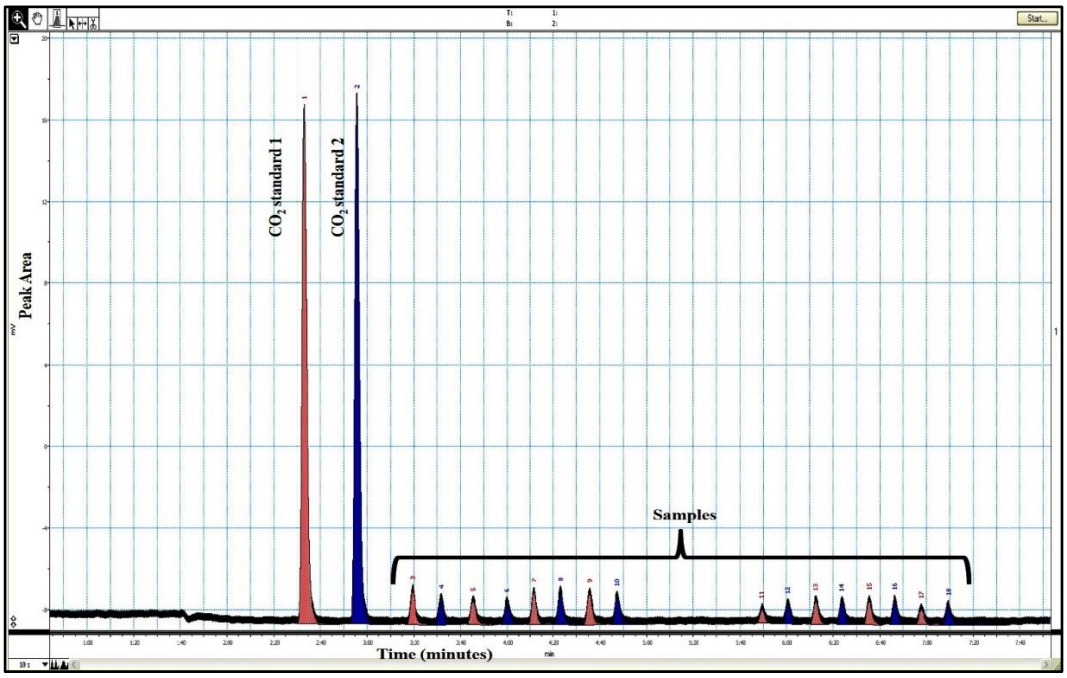


Figure 3.9 Servomex Gas Analyser's chromatographic profile of CO_2 standard (8.31±0.17 %) and sample gas peaks

3.6 Determination of fruit quality parameters

3.6.1 Physiological loss of weight (PLW)

The experiment fruit were weighed before transferring into storage and the initial fruit weight was recorded. After the end of the respective storage period, the fruit were weighed again and recorded as final weight. The PLW of the fruit was then calculated using the following formula and expressed as a percentage.

$$\text{PLW (\%)} = \frac{\text{Initial weight (kg) - Final weight (kg)} \times 100}{\text{Initial weight (kg)}}$$

3.6.2 Fruit firmness

The fruit firmness was determined using the Texture Analyser (TA Plus, Ametek Lloyd Instruments Limited, UK) (Figure 3.10) following the procedure detailed by Zaharah (2011). The texture analyser is fitted with the horizontal square base platform (15×15 cm) and interfaced with Nexygen® version 4.6 software installed on the desktop computer. A 5/16" (8.0 mm) Magnus-Taylor probe, equipped with 500 N load cell punctured the flesh of peeled portion of the fruit, to a sample depth of 7

mm at the test speed 100 mm s^{-1} and trigger force 5 Newton (N). Each experiment fruit was punctured on both sides along the equatorial region. The average value of both sides was taken as the firmness of that fruit. The fruit firmness was expressed as Newton (N).

Figure 3.10 'Texture Analyser' used to determine the fruit firmness

3.6.3 Soluble solid concentration (SSC), titratable acidity (TA) and SSC: TA

The juice was extracted from the peeled experiment fruit using the fruit juicer (Breville - Froojie Fountain Juicer, Sydney, Australia). The SSC of the extracted fruit juice sample was then determined using an infrared digital refractometer (Atago – Palette PR 101, Atago Co., Tokyo, Japan) and was expressed as % Brix.

TA of the same freshly extracted juice sample was estimated by the titration method. The juice sample (10 mL) was diluted with 20mL distilled water (dH_2O). The 5mL aliquot of diluted fruit juice sample was then titrated against 0.1N sodium hydroxide (NaOH) solution using burette, after adding 2-3 drops of phenolphthalein indicator. The change of aliquot colour to pale pink colour was considered endpoint and reading of the volume of NaOH used-up was noted at this point. The TA was then calculated using the following formula and expressed as per cent malic acid (Sadler and Murphy, 2010).

$$\text{Malic Acid (\%)} = \frac{\text{meq factor} \times \text{Vol. of NaOH used (mL)} \times \text{Vol. of titrant (mL)} \times 100}{\text{Vol. of juice (mL)} \times \text{Vol. of an aliquot (mL)}}$$

where,

meq factor = milli equivalent factor (Malic acid = 0.0067)

Vol. of NaOH used = reading on the burette

Vol. of titrant = 30 mL (final volume of diluted juice)

Vol. of juice = 10 mL

Vol. of aliquot = 5 mL

SSC: TA was calculated by dividing the values of SSC (% Brix) with the corresponding values of TA (%).

3.6.4 Fruit sampling

A sector of flesh portion was cut from each of the peeled fruits of every experiment unit and then chopped into small dices. All the chopped fruit sample was pooled, and the pulp samples were weighed, packed and stored at -20 °C refrigerator for respective qualitative analysis. The fruit quality parameters such as individual sugars, organic acids, total phenols, ascorbic acid and total antioxidant capacity were then analysed from the samples after thawing.

3.6.5 Individual sugars and organic acids

3.6.5.1 Preparation of sample

The pulp sample (5 g) extracted by the procedure detailed in Section 3.6.4 was added to 50 mL of degassed Milli-Q water and homogenised using homogeniser (Heidolph DIAX 900, Heidolph Co. Limited, Germany). Milli-Q water was obtained by filtering distilled water through the ultrapure water purification system (Simplicity®, Millipore Co. Limited, USA). The Milli-Q water was then degassed by passing through a 0.45 µm nylon filter and vacuum pump. The homogenised sample was centrifuged at 12,880×*g* for 15 min using a refrigerated centrifuge at 4°C

(Eppendorf 5810 R, Germany). After the centrifugation, an aliquot of the supernatant was drawn using a syringe and filtered through a 0.22 µm nylon syringe filter (Thermo Fisher Scientific Australia Pty Limited, Australia) to load into 1 mL clear glass vials (Thermo Fisher Scientific Australia Pty Limited, Australia). The glass vials are then tightly capped and were used for the determination of individual sugars and organic acids using high-performance liquid chromatography (HPLC) system.

Figure 3.11 High-performance liquid chromatography (HPLC) system used for individual sugars and organic acid analysis

3.6.5.2 HPLC system

The reverse-phase HPLC (Figure 3.11) was used to analyse the levels of individual sugars and organic acids. HPLC system (Waters 1525, Milford Corporation, USA) was equipped with Dual λ absorbance detector (Waters 2487, Milford Corporation, USA) or Refractive Index (RI) detector (Water 2414, Milford Corporation, USA) and autosampler (Waters 717plus, Milford Corporation, USA). The sample glass vials prepared as per the procedure detailed in Section 3.6.4.1 were arranged on the carousel and placed in the autosampler, which was maintained at 25 °C. The sugars and organic acids were separated and analysed isocratically on the respective columns (Table 3.3). The columns were preceded by Cation H Bio-Rad Micro-Guard® column (30 × 4.6 mm) (Bio-Rad Laboratories Inc., USA). The separation column and guard column were maintained at 60 °C for sugars and 45 °C for organic acids separation. Individual organic acids were detected by Dual λ UV absorbance detector (Water 2487, Milford Corporation, USA) at 214 nm and individual sugars were detected by Refractive Index (RI) detector (Water 2414,

Milford Corporation, USA). The standard chemicals as mentioned in Annexure 1 were analysed to identify the retention times of the chromatographic peaks for the specific compound. The peaks of the samples were then compared with retention times (Table 3.4, Figure 3.12 and 3.13) of the pure standard compounds and analysed accordingly, using Breeze®2 software version 6.20 (Waters, Milford Corporation, USA) installed on the desktop computer, which was interfaced with HPLC system.

Table 3.3 Details of the columns used in HPLC systems to analyse levels of individual sugars and organic acids

	Column details	**Mobile phase and flow rate**	**Contents analysed**
Sugars	Bio-Rad Aminex® HPX-87C Fast Carbohydrate column (100×7.8 mm) Particle size: 9 µm	Degassed Milli-Q water @ 0.5-0.6 mL min^{-1}	1. Fructose 2. Glucose 3. Sucrose 4. Sorbitol
Organic acids	Bio-Rad Aminex® HPX-87H column (300 × 7.8 mm) Particle size: 9 µm	0.01 N H_2SO_4 @ 0.5-0.6 mL min^{-1}	1. Citric acid 2. Fumaric acid 3. Malic acid 4. Succinic acid 5. Tartaric acid

Table 3.4 Elution order and retention times of different sugars and organic acids identified using HPLC system

	Elution order	Compound	Retention peak (min)	Peak range (min)
Sugars	1	Sucrose	4.115	3.73 – 4.50
	2	Glucose	4.842	4.52 – 5.40
	3	Fructose	6.637	5.63 – 8.25
	4	Sorbitol	14.015	11.61 – 16.76
Organic acids	1	Citric Acid	7.934	7.58 – 8.23
	2	Tartaric Acid	8.485	8.18 – 8.87
	3	Malic Acid	9.470	9.14 – 10.23
	4	Succinic Acid	11.630	11.04 – 12.42
	5	Fumaric Acid	14.051	12.57 – 16.07

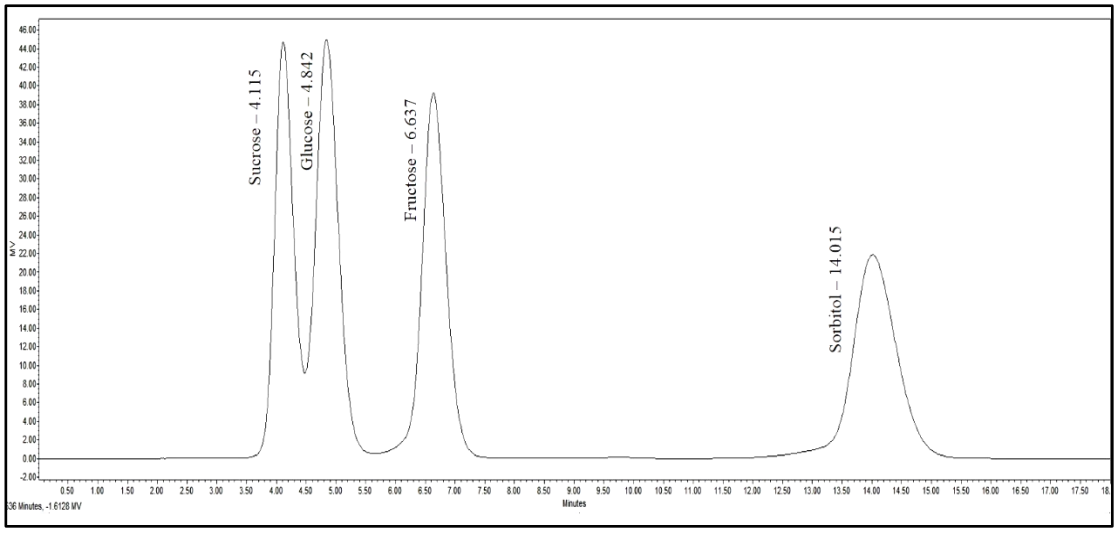


Figure 3.12 HPLC chromatographic profiles of individual standard sugars

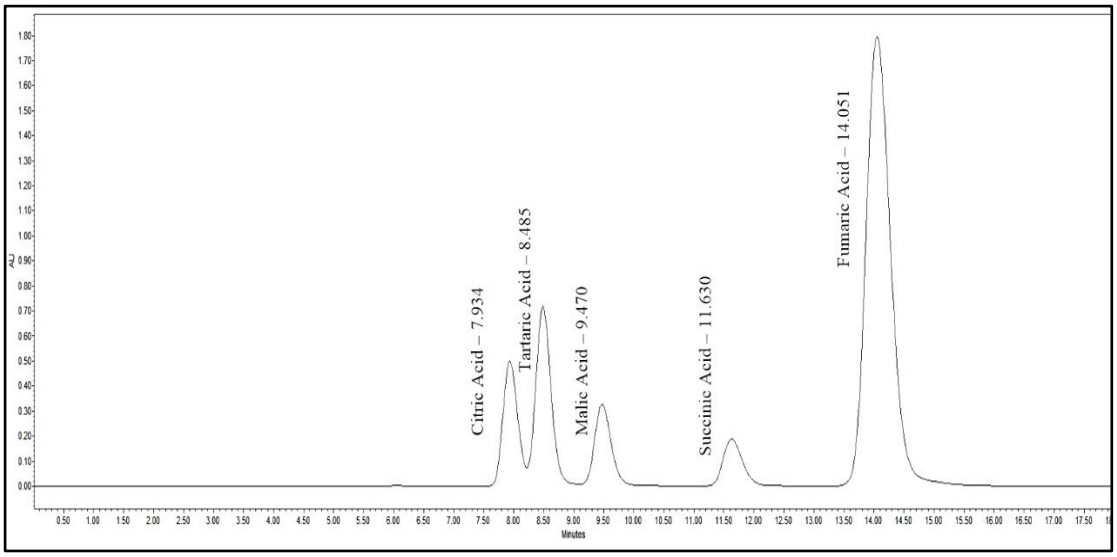


Figure 3.13 HPLC chromatographic profiles of individual standard organic acids

3.6.6 Total phenols

The level of total phenols in the fruit pulp was determined using Folin-Ciocalteu reagent, according to the procedure described by Vithana et al. (2018), with some modifications from the method explained by Robles-Sánchez et al. (2009) (Figure 3.14). The 20 g of representative pulp sample of the experimental unit, extracted using the procedure explained in section 3.6.4, was added to 15 mL of 80% (v/v) methanol (CH_3OH) solution and homogenised using Heidolph DIAX 900 homogeniser (Heidolph Co. Limited, Germany). The homogenised sample was then sonicated for 15 min (Soniclean Pvt. Limited, Germany) at a 42 kHz frequency. Following the sonication, the samples were centrifuged for 15 min at 10,000×g at 4 °C using refrigerated centrifuge (Eppendorf 5810 R, Germany) and the supernatant solution was separated. The re-extraction was done from the residue following a similar procedure. The supernatants were then pooled and then the volume was made to 60 mL by distilled water. The 50 µL aliquot was then added to 3.0 mL distilled water and 250 µL two-fold diluted Folin-Ciocalteu reagent (1:2 = Folin-Ciocalteu reagent: CH_3OH) and kept it in dark for 5 min to react. Then, 250 µL 7% sodium carbonate (Na_2CO_3) was added and volume made to 5 mL by distilled water (1450 µL). The mixture was kept for an additional 90 min in dark and after then the absorbance was measured at 750 nm wavelength using UV/VIS spectrophotometer

(Jenway spectrophotometer Model 6405, UK). The total phenolic content was calculated with respect to the standard curve of pure gallic acid and was expressed in g gallic acid equivalents (GAE) kg^{-1} fresh weight basis.

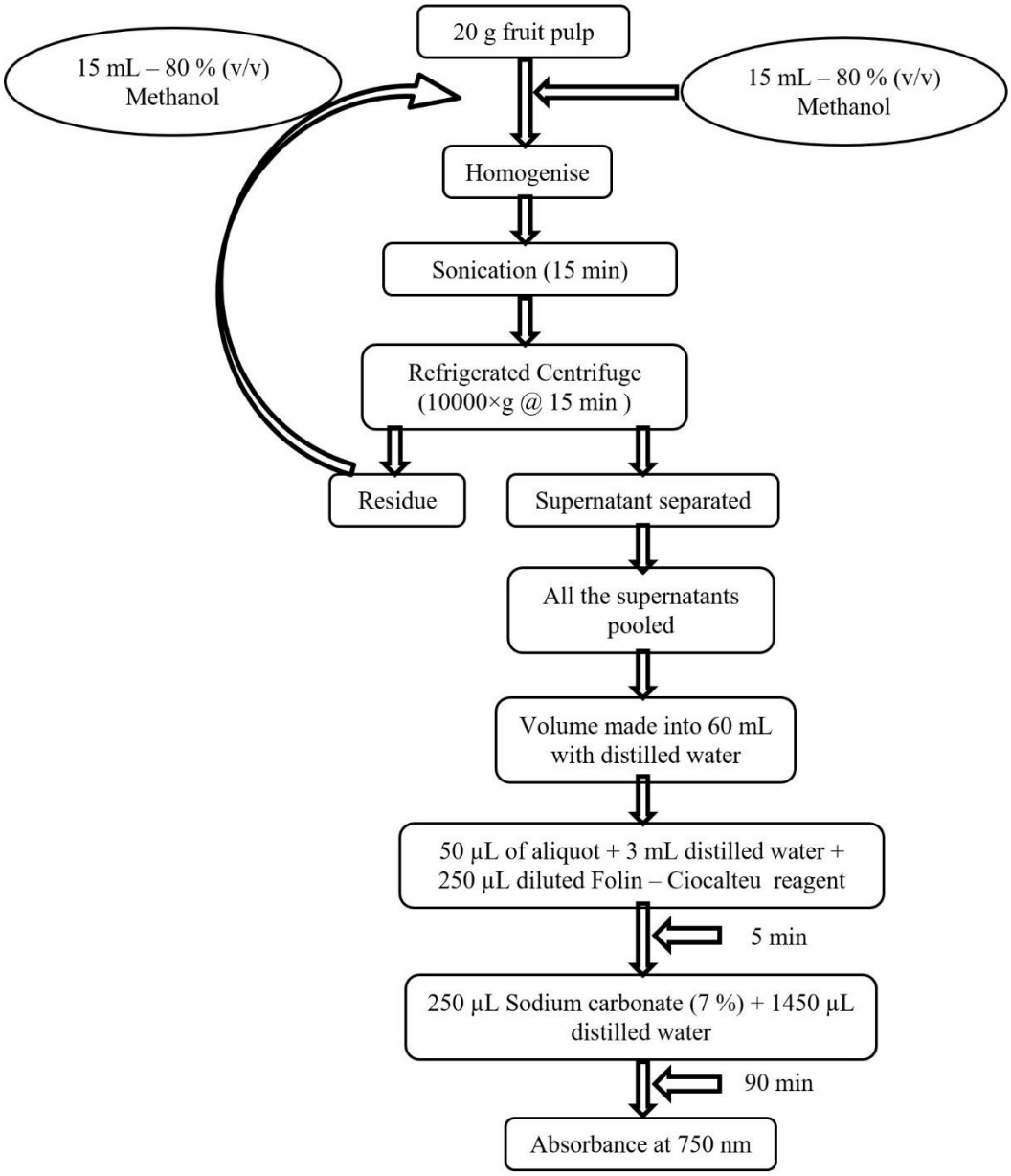


Figure 3.14 Flow chart for extraction and estimation of total phenols in pulp (Vithana, 2017).

3.6.7 Ascorbic acid

The ascorbic acid levels in the fruit pulp were estimated using the method explained by Malik and Singh (2005) (Figure 3.15). The 5 g fruit pulp sample, obtained by the

sampling procedure mentioned in section 3.6.4, was mixed with 20 mL of 6% metaphosphoric acid solution (60 g metaphosphoric acid + 1.8 g EDTA (Ethylenediaminetetraacetic acid) + 1 L distilled water) and homogenised. The homogenised sample was then centrifuged at 5000×g for 20 min using refrigerated centrifuge (Eppendorf 5810 R, Germany) at 4°C. The 400 µL aliquot of the supernatant was then mixed 200 µL 3 % metaphosphoric acid solution, 1400 µL distilled water and 200 µL diluted Folin-Ciocalteu reagent (1:5 = Folin-Ciocalteu reagent: distilled water) and kept in dark for 10 min. The absorbance of the mixture was recorded at 760 nm using UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK). The ascorbic acid concentration was calculated using the curve of standard L-ascorbic acid and was expressed as g kg^{-1} fresh weight (FW) basis.

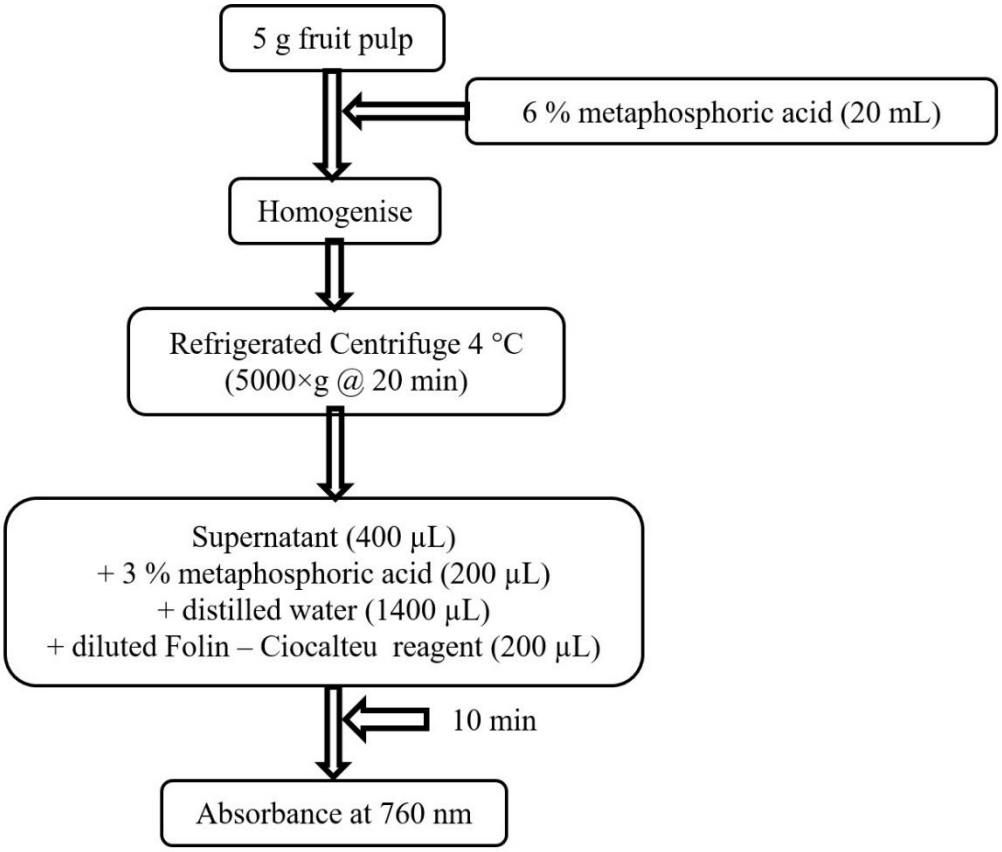


Figure 3.15 Flowchart for determination of ascorbic acid in fruit pulp (Vithana, 2017)

3.6.8 Total antioxidant capacity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method described by Brand-Williams et al. (1995) and slightly modified by Vithana et al. (2018) was used to determine the total antioxidant capacity in the fruit pulp sample. The pulp sample (1 g) obtained by the sampling procedure explained in section 3.6.4 was mixed with 10 mL of extraction buffer (2 mM sodium fluoride (NaF) in 80 % (v/v) CH_3OH) and homogenised (Heidolph DIAX 900, Heidolph Co. Limited, Germany). The extraction buffer was prepared by dissolving 84 mg NaF in 1 L 80 % (v/v) CH_3OH. The homogenised samples were then centrifuged at 10,000×g and 4 °C using refrigerated centrifuge (Eppendorf 5810 R, Germany) for 20 min. The DPPH stock solution for the analysis was prepared by dissolving 24 mg DPPH in 100 mL CH_3OH (99.90 %). The daily working solution was prepared by diluting the stock solution in the methanol while adjusting the absorbance value to 1.10 at a 515 nm wavelength on UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK). The absorbance value of the daily working solution was adjusted by adding CH_3OH in case > 1.10 and adding stock solution when absorbance value < 1.10. An aliquot of the supernatant was mixed with the 1900 µL daily working solution and the spectrophotometer reading of the absorbance was recorded after keeping the mixture in dark for 15 min. The volume of aliquots was adjusted until an absorbance value between 0.6 and 0.7 was obtained. The volume of the aliquot was increased when the value was > 0.700 and reduced when < 0.600. The total antioxidant capacity of the sample was calculated with respect to the standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (97 %)). The calculated value of the total antioxidant capacity was expressed as µM kg^{-1} Trolox fresh weight basis (FW).

3.7 Statistical analysis of data

GenStat software version 14.0 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) was used to statistically analyse all the data obtained from different experiments. The results were presented as means ± standard errors (SE) of the mean. Based on the experiment details, the data were analysed by one-way or two-way analysis of variance (ANOVA). The least significant difference (LSD) was determined following F-test with 5 % error probability. Duncan multiple comparison tests were used to comparing the treatment means.

CHAPTER 4

Effect of different formulations of novel ethylene antagonists on the ethylene production and fruit quality of cold-stored Cripps Pink apples

Abstract

The flexibility to treat the fruit with ethylene antagonists as an aqueous solution would enable to apply the postharvest treatments as a fruit dip or line spray during the packing line. The efficacy of aqueous dip treatments of the two novel ethylene antagonists namely 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) and fumigation of BC, NC and 1-methylcyclopropene (1-MCP) in reducing the rates and delaying the onset of ethylene and respiration as well as in maintaining the fruit quality of cold-stored Cripps Pink apple fruit were investigated. The apple fruit were fumigated with 1 µM BC, 1 µM NC, or 1 µL L^{-1} 1-MCP for 18 h or dipped in 20 L aqueous solution of 5 % ethanol alone, 2 µM BC + 5 % ethanol or 2 µM NC + 5 % ethanol for 5 min (at 20±2 °C). The untreated fruit was considered as control. The experiment was laid in completely randomised design and each treatment was replicated four times. Each experimental unit consisted of fifteen fruit. Both the fumigation and dip treatments of ethylene antagonists retarded the rates of ethylene production, reduced PLW as well as maintained high firmness, total phenols and titratable acidity in the fruit when compared to control and ethanol treatments during 100 and 150 days of storage. The BC and NC fumigation treatments performed better than respective dip treatments in retarding ethylene and in maintaining the fruit quality of Cripps Pink apple during 100 and 150 days of cold storage.

4.1 Introduction

Fruit ripening is a natural process involving numerous anabolic and catabolic reactions. It is considered the final stage of fruit development associated with several interdependent physiological and biochemical changes, which ultimately lead to senescence and deterioration. The fruit ripening is an irreversible process typically include processes such as starch hydrolysis, fruit softening, depletion of phenolic compounds and development of aroma volatile compounds in the fruit (Tucker, 2012).

The plant hormone ethylene has been recognised and proved to promote fruit ripening process (Burg and Burg, 1962). In case of the climacteric fruits such as apple, pear and mango the ripening process is accelerated with exposure of external ethylene gas and it also initiates internal ethylene production in an autocatalytic manner (Imaseki, 1991). The apple fruit is classified under the group of fruit, which are highly sensitive to external ethylene exposure and produces large amounts of ethylene during the ripening process. The levels of internal ethylene production and the storage conditions significantly affect the postharvest quality and storage life of the apple fruit (Abeles et al., 1992).

Proper postharvest handling and optimum storage conditions can significantly extend the storage life of fruits while maintaining commercially acceptable quality. Storing the fruit at the low temperatures enhance the storage life by reducing rates of respiration and ethylene production (Gross et al., 2016). The fruit treatment with 1-substituted CP inhibits ethylene action by blocking the ethylene receptors as well as by interfering the signal perception associated with ripening-associated genes (Klee, 2004; Apelbaum et al., 2008). Among all the CP, 1-MCP is well recognised as relatively stable CP at the gaseous state and as an effective ethylene action inhibitor. 1-MCP has been widely used in the horticulture industry to prolong the storage life of different fruit and vegetables (Valero et al., 2016). The efficacy of 1-MCP fumigation treatments varies with the difference in concentrations, treatment duration, fruit species, cultivars, storage environment and maturity stage (Schotsmans et al., 2009, Escribano et al., 2017). The boiling point of 1-MCP is as low as 0°C and is unstable liquid at room temperatures (Sisler et al., 2006). Hence, the postharvest application of 1-MCP to the fruit is usually being done through fumigation and in sealed storage rooms or containers. The ability of ethylene antagonist to treat as an aqueous solution would open prospects of postharvest treatments as a fruit dip or line spray during the packing line. It would also allow application in the situations where sealed rooms are not available or when there is a delay in filling storage room to its maximum capacity (Argenta et al., 2007). There exists need to explore possibilities to develop compounds which could address the limitations of 1-MCP with ethylene antagonistic effect equivalent to 1-MCP. Keeping the above points in view, the following experiment was designed to study the efficiency of two novel ethylene antagonist (BC and NC) treatment as fumigation and aqueous dip in lowering the rates of respiration and

ethylene production. The efficacy of these treatments in maintaining the postharvest fruit quality during two different cold storage durations was also investigated. It was hypothesised that both fumigation and dip treatments of new ethylene antagonists will effectively antagonise ethylene action and retard ripening-associated changes promoted by ethylene, during both 100- and 150-days cold storage.

4.2 Material and methods

4.2.1 Fruit and experiment conditions

The Cripps Pink apple fruit used in this experiment were obtained from Balingup (34°13' S latitude, 116°08' E longitude) at commercial harvest (fruit firmness 66.76±3.84 N; SSC 15.03±0.04 %; TA 0.82±0.04 %) on 7th May 2018. The fruit was collected from 23 years old trees grafted on MM106 rootstock. The trees were planted in North-South orientation with the spacing of 4.5 m (within rows) and 3m (between rows) and trained as modified central leader. After the harvest, the fruit were dipped in an aqueous solution of 'Magnate 750WG' (a.i. 750 g L^{-1} Imazalil) @ 0.68 g L^{-1}, 'Stopit' (a.i. 160 g L^{-1} liquid calcium chloride ($CaCl_2$) @ 15 mL L^{-1} and DPA (diphenylamine) @ 5 mL L^{-1} to protect them from postharvest diseases and disorders during storage. The fruit were air-dried in open, till there are no water droplets left on the fruit surface, before placing them in soft board trays and then packed in corrugated cardboard boxes. The packed fruit were transported immediately using an air-conditioned vehicle to Curtin Horticulture Research Laboratory, Perth.

Relatively uniform sized fruits free from bruises, injuries, pest and diseases were chosen for the experiment. The fruit were fumigated with ethylene antagonists (BC, NC and 1-MCP) as explained in Chapter 3, Section 3.3.1, keeping sixty fruit per drum. The other set of fruit were dipped in ethanol (5 %) or ethylene antagonist (NC and BC) solutions as explained in Chapter 3, Section 3.3.2 while making sure that whole fruit was dipped in the solution for 5 min. Maintaining the constant concentration of pure 1-MCP solution at room temperature is difficult as it is highly volatile in the liquid state. The fruit untreated were used as a control. The treated fruit were then packed in corrugated cardboard boxes with softboard trays with respect to the treatment. The experiment was laid in completely randomised block design and each treatment was replicated four times, with fifteen fruit per replication. Each box was packed with sixty

(fifteen fruit replicated four times) fruit with respect to the treatments. All the boxes were divided into two lots, one lot meant to be stored for 100 days and another lot for 150 days in the cold storage at Curtin Horticulture Research Laboratory, Perth. The fruit were taken out of cold storage after completion of designated storage period to analyse physiological and quality parameters of the fruit.

4.2.2 Determination of fruit physiological parameters

On completion of the designated cold storage period, two fruit per experimental unit were randomly selected to determine the rate of ethylene production and respiration.

4.2.2.1 Rate of ethylene production

The selected two fruit were incubated in 1 L air-tight glass jar for one hour at room temperature (20±2°C). A gas sample of 1 mL was drawn through rubber septum from the headspace of an individual glass jar and injected into GC (6890N Network GC system; Agilent Technology, CA, USA) in order to determine the amount of ethylene produced by the sealed fruit. The rate of ethylene production was determined daily until distinct climacteric peak was achieved. The detailed procedure and calculations for estimation of ethylene production are explained in Chapter 3, Section 3.5.1. The rate of ethylene production is expressed as µmol C_2H_4 kg^{-1} h^{-1}.

4.2.2.2 Rate of respiration

The selected fruit were enclosed in a 1 L air-tight glass jar for one hour (at 20±2°C). A two mL gas sample was drawn through the rubber septum from the headspace of individual glass jars and injected into Infrared gas analyser (Servomex Gas Analyser, 1450 Food Package Analyser, Servomex Limited, UK) to determine levels of carbon dioxide. The rate of respiration in the fruit was calculated based upon the carbon dioxide evolved and the calculations have been explained in detail in Chapter 3, Section 3.5.2. The rate of respiration in apple fruit is expressed as mmol CO_2 kg^{-1} h^{-1}.

4.2.3 Determination of fruit quality parameters

4.2.3.1 Physiological loss of weight (PLW)

Just before transferring fruit into the cold storage the weight of fifteen fruit per replication was recorded as initial weight. After completion of the designated cold storage period, the fruit were weighed again and recorded as final weight. The PLW was calculated using formula as explained in Chapter 3, Section 3.6.1. The PLW of apple fruit is expressed as %.

4.2.3.2 Fruit firmness

On completion of the designated cold storage period, the fruit firmness of ten randomly chosen fruit per replication was determined using Texture Analyser (TA Plus, Ametek Lloyd Instruments Limited, UK). The detailed procedure to determine fruit firmness and the calculations were explained in Chapter 3, Section 3.6.2. The fruit firmness calculated is expressed as newtons (N).

4.2.3.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA

The SSC, TA and SSC: TA ratio was determined from the pooled juice samples extracted from slices taken from thirteen fruits per replication. The SSC of the juice samples was determined using an infrared digital refractometer (Atago- Palette PR 101, Atago Co., Tokyo, Japan). The TA of the fruit juice sample was determined using the titration method. The SSC, TA and SSC: TA ratio of the fruit juice samples were calculated following the methods detailed in Chapter 3, Section 3.6.3. The SSC is expressed as % Brix and TA as % malic acid.

4.2.3.4 Individual sugars and organic acids

The pulp samples extracted from thirteen fruit per replication were used to prepare samples to estimate individual sugars and organic acid levels. The levels of individual sugars (glucose, fructose, sucrose and sorbitol) and organic acids (malic acid, succinic acid, fumaric acid, tartaric acid and citric acid) were estimated using reverse-phase HPLC system (Waters 1525, Milford Corporation, USA). The individual organic acids were determined using Dual λ UV absorbance detector (Water 2487, Milford Corporation, USA) at 214nm, while Refractive Index (RI) detector (Water 2414, Milford Corporation, USA) was used to determine individual sugars. The detailed description of instruments, procedures to prepare and run the samples were explained

in Chapter 3, Section 3.6.5. The levels of sugars and organic acids are expressed as g kg^{-1}.

4.2.3.5 Total phenols

The total phenol levels in the fruit samples were estimated using the Folin-Ciocalteu reagent method following the procedure detailed in Chapter 3, Section 3.6.6. The curve drawn using standard gallic acid was used to calculate the levels of total phenolic content and expressed as g GAE kg^{-1} fresh weight basis.

4.2.3.6 Ascorbic acid

The levels of ascorbic acid in the fruit pulp samples were determined following the procedure detailed in Chapter 3, Section 3.6.7 using UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK). The curve drawn using standard L-ascorbic acid was used to calculate levels of ascorbic acid and expressed as g kg^{-1} fresh weight basis.

4.2.3.7 Total antioxidant capacity

The total antioxidant capacity in the fruit pulp samples was determined using UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK) following the DPPH method. The details about the sample preparation and estimation procedure was explained in Chapter 3, Section 3.6.8. The curves drawn using standard Trolox was used to calculate levels of total antioxidant activity and expressed as µM kg^{-1} Trolox fresh weight basis.

4.2.4 Statistical analysis

All the data recorded was analysed statistically using *GenStat* software version 14.0 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The results were presented as means ± standard errors (SE) of the means. The data of fruit physiological parameters were analysed by one-way analysis and all other data were analysed by two-way analysis of variance (ANOVA) with ethylene antagonist treatments and storage duration as two factors. The F-test was used to determine the least significant

difference (LSD) with 5 % error probability. The treatment means were compared using Duncan multiple comparison tests.

4.3 Results

4.3.1 Fruit physiological parameters

4.3.1.1 Ethylene (C_2H_4) production

The mean C_2H_4 climacteric peak rates in Cripps Pink fruit were significantly reduced with ethylene antagonist treatments when compared to control and ethanol treatments, in both 100 and 150 days of cold storage (Figure 4.1). The fruit fumigated with 1-MCP exhibited lowest mean ethylene climacteric peak rates in both 100 and 150 days cold-stored Cripps Pink fruit (0.02 and 0.03 µmol kg^{-1} h^{-1}, respectively) when compared to all other treatments (Table 4.1). The fruit fumigated with BC and NC showed lower values of mean C_2H_4 climacteric peak rates when compared to their respective fruit dip treatments in ethylene antagonist solutions during both 100 and 150 days of cold storage (Table 4.1).

The onset of C_2H_4 climacteric peak was significantly delayed in the Cripps Pink fruit fumigated with 1-MCP during both 100 and 150 days of cold storage (9.50 and 8.75 days, respectively) (Table 4.1). Next to the 1-MCP treatment the Cripps Pink fruit fumigated with BC (7.50 days) exhibited delayed ethylene climacteric peak during 100 days of cold storage. But in case of the fruit cold-stored for 150 days, there was no significant difference in the ethylene climacteric peak onset among the treatments other than 1-MCP (Table 4.1).

4.3.1.2 Respiration (CO_2 production)

Following the 100 days of cold storage, the levels of mean respiratory climacteric peak rate were significantly lowest in the fruit fumigated with 1-MCP (0.51 mmol kg^{-1} h^{-1}), but no significant difference was found among other treatments. The fruit fumigated with ethylene antagonists BC, NC and 1-MCP exhibited significantly lowest (0.69 mmol $kg^{-1}h^{-1}$, 0.65 mmol $kg^{-1}h^{-1}$ and 0.54 mmol $kg^{-1}h^{-1}$, respectively) respiratory climacteric peak rate values when compared to control, ethanol and ethylene antagonist dip treatments in 150 days cold-stored Cripps Pink fruit (Table 4.1).

The onset of the respiratory climacteric peak was not significantly affected by any of the treatments applied, in 100 days cold-stored fruit. In case of the 150 days cold-stored fruit, the fumigation treatment with ethylene antagonists (BC, NC and 1-MCP) significantly delayed respiratory climacteric peak onset when compared to the control fruit and the fruit dipped in ethanol and ethylene antagonist solutions (Table 4.1).

Table 4.1 Effect of the different formulations of ethylene antagonists on the climacteric peak onset (days) and peak rate of ethylene (µmol kg^{-1} h^{-1}) and respiration (mmol kg^{-1} h^{-1}) of Cripps Pink apple fruit stored in cold storage

	Storage period (days)			
Treatment	100		150	
	C_2H_4 climacteric peak onset	C_2H_4 climacteric peak rate	C_2H_4 climacteric peak onset	C_2H_4 climacteric peak rate
Control	3.50±0.25a	2.59±0.12e	4.50±0.25a	2.91±0.21d
Ethanol	4.50±0.75ab	2.30±0.10de	3.50±0.25a	3.32±0.22d
BC Fumigation	7.50±1.03cd	1.03±0.13b	4.75±0.41a	1.70±0.08bc
BC Dip	3.50±0.25a	1.93±0.14c	4.00±0.00a	2.10±0.12c
NC Fumigation	5.00±0.61abc	1.29±0.03b	5.00±0.00a	1.37±0.03b
NC Dip	6.25±1.02bc	2.12±0.18cd	4.25±0.22a	1.85±0.11c
1-MCP	9.50±0.43d	0.02±0.02a	8.75±1.08b	0.03±0.02a
LSD ($P \leq 0.05$)	2.45	0.34	1.61	0.43
	Respiration climacteric peak onset	Respiration climacteric peak rate	Respiration climacteric peak onset	Respiration climacteric peak rate
Control	3.25±0.41	0.75±0.01b	4.00±0.00a	0.88±0.06b
Ethanol	4.00±0.00	0.64±0.02b	5.00±0.50abc	0.88±0.07b
BC Fumigation	4.50±1.09	0.70±0.03b	6.25±0.22c	0.69±0.02a
BC Dip	4.25±0.54	0.70±0.07b	4.00±0.00a	0.88±0.07b
NC Fumigation	5.00±0.87	0.66±0.04b	6.00±0.00bc	0.65±0.01a
NC Dip	4.25±0.22	0.71±0.05b	4.75±0.65ab	0.88±0.04b
1-MCP	4.25±0.22	0.51±0.01a	5.50±0.43bc	0.54±0.04a
LSD ($P \leq 0.05$)	ns	0.13	1.22	0.18

ns = non-significant, n = 4 replicates (2 fruit per replication), mean ± SE. The Duncan's multiple range test at ($P \leq 0.05$) was used to test the mean separation for significant analysis of variance within the columns. Mean values followed by a similar letter are not significantly different within the columns. Mean values without letters within columns are non-significant. The data of 100- and 150-days cold storage were analysed separately.

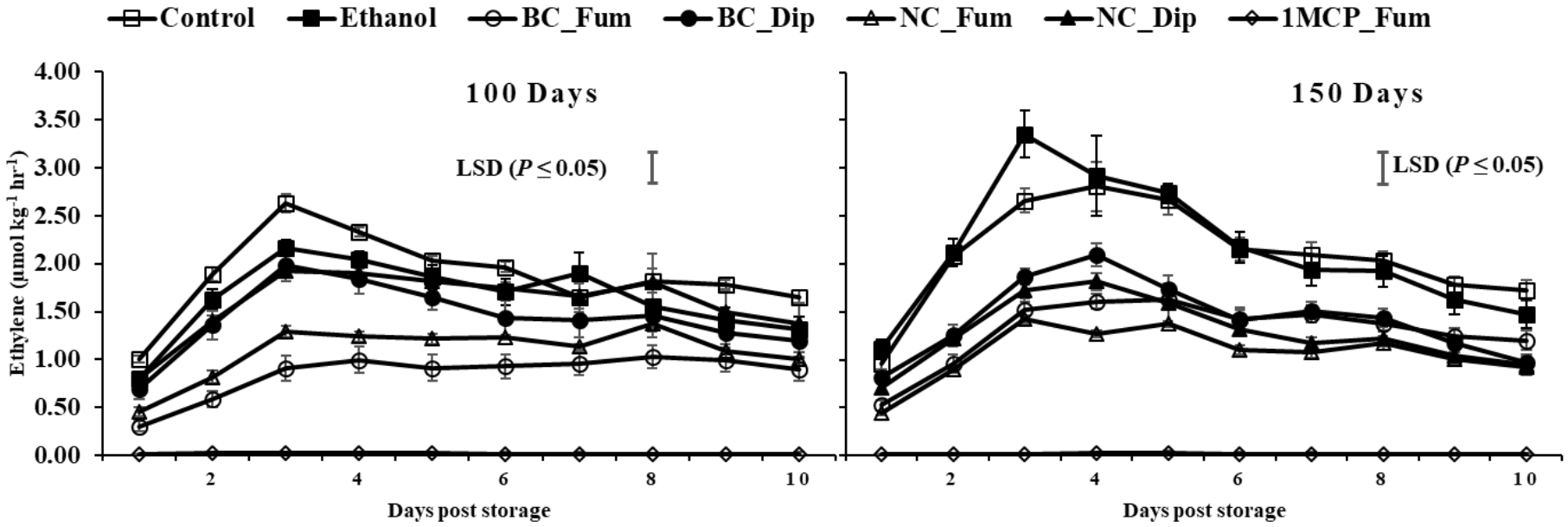


Figure 4.1 Changes in rates of ethylene production due to ethylene antagonist treatments applied as dip and fumigation formulations (T) days past storage (D) in Cripps Pink apple fruit stored for 100 and 150 days. The vertical bars in the graph represent SE of the mean values and are not visible when values are smaller than the symbol. n= 4 replicates (2 fruit per replication). LSD ($P \leq 0.05$) T= 0.10, D= 0.12, TXD= 0.32 for 100 days and T= 0.11, D= 0.13, TXD= 0.34 for 150 days.

4.3.2 Fruit quality parameters

4.3.2.1 Physiological loss of weight (PLW)

The Cripps Pink fruit fumigated with 1-MCP, NC and BC exhibited significantly lower mean PLW (2.37 %, 2.64 % and 2.55 %, respectively) when compared to the remaining treatments. The control fruit exhibited significantly highest mean PLW (Table 4.2). The fruit cold-stored for 150 days exhibited significantly higher mean PLW (3.17 %) when compared to 100 days cold-stored fruit (2.28 %) (Table 4.2). The interaction effect between the storage period and ethylene antagonist treatments on the PLW was non-significant.

4.3.2.2 Fruit firmness

The mean firmness of the Cripps Pink fruit fumigated with ethylene antagonists 1-MCP, BC and NC was significantly higher (57.10 N, 50.97 N and 50.73 N, respectively) than the control fruit and the fruit dipped in ethanol or ethylene antagonist solutions (Table 4.2). The firmness of the fruit cold-stored for 150 days (49.60 N) was significantly lower than the fruit stored for 100 days (50.73 N) (Table 4.2). There was no significant interaction effect between the storage period and ethylene antagonist treatments on the firmness of the cold-stored fruit.

Table 4.2 Effect of the different formulations of ethylene antagonists on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Cripps Pink apple fruit stored in cold storage.

	Storage period (days)		
Treatment	100	150	Mean (T)
	Physiological loss of weight (PLW)		
Control	2.36±0.19	3.83±0.18	3.09C
Ethanol	2.33±0.13	3.42±0.10	2.88BC
BC Fumigation	2.25±0.13	2.86±0.09	2.55AB
BC Dip	2.22±0.09	3.28±0.16	2.75BC
NC Fumigation	2.29±0.14	2.99±0.13	2.64AB
NC Dip	2.32±0.11	3.26±0.23	2.79BC
1-MCP	2.18±0.13	2.56±0.10	2.37A
Mean (D)	2.28A	3.17B	
LSD ($P \leq 0.05$)	T=0.34	D=0.18	TXD=ns
	Fruit firmness		
Control	48.36±0.74	46.87±0.48	47.61A
Ethanol	48.61±0.55	47.60±0.77	48.10A
BC Fumigation	51.72±0.24	50.23±0.48	50.97B
BC Dip	48.64±0.43	46.83±0.75	47.73A
NC Fumigation	50.98±0.57	50.47±0.86	50.73B
NC Dip	49.23±0.24	48.62±1.30	48.93A
1-MCP	57.59±0.69	56.61±0.63	57.10C
Mean (D)	50.73B	49.60A	
LSD ($P \leq 0.05$)	T=1.58	D=0.84	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (15 fruit (PLW) and 10 fruit (fruit firmness) per replication), mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The upper case letter was used for treatment and storage period means.

4.3.2.3 Soluble solid concentration (SSC), titratable acidity (TA) and SSC: TA

The Cripps Pink fruit dipped in the ethanol (16.09 %) or ethylene antagonist BC (16.20 %), NC (15.99 %) solutions exhibited significantly lower SSC values when compared to control and ethylene antagonists (NC and 1-MCP) fumigation treatments. The fruit fumigated with NC and 1-MCP showed significantly highest (16.44 % and 16.49 %, respectively) SSC values (Table 4.3). The SSC values were significantly highest in the fruit stored for 150 days (16.25 %) (Table 4.3). The interaction effect between storage period and ethylene antagonist treatments on SSC values was significant but followed no specific trend (Table 4.3).

The mean TA values of the Cripps Pink fruit fumigated with 1-MCP were significantly highest (0.70 %) followed by the fruit fumigated with NC (0.63 %). The control fruit exhibited the lowest mean TA value (0.58 %) (Table 4.3). The TA values of the fruit cold-stored for 150 days (0.59 %) were significantly lower than fruit stored for100 days (0.64 %). There was a significant interaction effect of storage period and ethylene antagonist treatments on TA values. The control fruit stored for 150 days showed the lowest value (0.54 %), while fruit fumigated with 1-MCP and stored for 100 days exhibited significantly highest TA value (0.78 %) (Table 4.3).

The Cripps Pink fruit fumigated with 1-MCP exhibited significantly lowest (23.77) mean SSC: TA values when compared to all other treatments, while control fruit showed the highest value (28.11) (Table 4.3). The mean SSC: TA value of 150 days cold-stored fruit was significantly higher (27.47) than 100 days stored fruit (25.39) (Table 4.3). A significant interaction effect between storage period and ethylene antagonist treatments was recorded and control fruit cold-stored for 150 days exhibited highest (29.99) SSC: TA value (Table 4.3)

Table 4.3 Effect of the different formulations of ethylene antagonists on the changes in the SSC (%), TA (%) and SSC: TA of the juice of Cripps Pink apple fruit stored in cold storage

	Storage period (days)		
Treatment	100	150	Mean (T)
	Soluble solids concentration (SSC)		
Control	16.33±0.02c	16.25±0.03bc	16.29D
Ethanol	15.95±0.02a	16.23±0.02bc	16.09B
BC Fumigation	16.25±0.04c	16.03±0.02a	16.14BC
BC Dip	16.15±0.02b	16.25±0.03c	16.20C
NC Fumigation	16.33±0.02c	16.55±0.03e	16.44E
NC Dip	16.03±0.02a	15.95±0.02a	15.99A
1-MCP	16.45±0.03d	16.53±0.04de	16.49E
Mean (D)	16.21A	16.25B	
LSD ($P \leq 0.05$)	T=0.07	D=0.04	TXD=0.09
	Titratable acidity (TA)		
Control	0.62±0.01def	0.54±0.01a	0.58A
Ethanol	0.63±0.01ef	0.55±0.01ab	0.59AB
BC Fumigation	0.62±0.01def	0.59±0.01cd	0.61B
BC Dip	0.61±0.01cde	0.58±0.01bc	0.60AB
NC Fumigation	0.61±0.01cde	0.65±0.01f	0.63C
NC Dip	0.61±0.01cde	0.61±0.01cde	0.61BC
1-MCP	0.78±0.01g	0.62±0.01def	0.70D
Mean (D)	0.64B	0.59A	
LSD ($P \leq 0.05$)	T=0.02	D=0.01	TXD=0.03
	SSC: TA		
Control	26.23±0.40bc	29.99±0.60e	28.11D
Ethanol	25.21±0.34b	29.39±0.52e	27.30CD
BC Fumigation	26.11±0.46bc	27.05±0.45cd	26.58BC
BC Dip	26.37±0.39bc	27.91±0.48d	27.14CD
NC Fumigation	26.65±0.33bcd	25.35±0.31b	26.00B
NC Dip	26.16±0.37bc	26.04±0.38bc	26.10B
1-MCP	21.00±0.27a	26.55±0.41bcd	23.77A
Mean (D)	25.39A	27.47B	
LSD ($P \leq 0.05$)	T=0.98	D=0.53	TXD=1.39

T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage period means

4.3.2.4 Individual sugars

The Cripps Pink fruit fumigated with BC (9.31 g kg^{-1}) and 1-MCP (9.20 g kg^{-1}) exhibited significantly higher levels of glucose along with control fruit (9.92 g kg^{-1}) compared all other treatments (Table 4.4). The fruit cold-stored for 150 days (6.79 g kg^{-1}) showed lower mean glucose levels compared to fruit stored for 100 days (8.88 g kg^{-1}) (Table 4.4). There was no significant effect of the storage period or ethylene antagonist treatments on the levels of fructose in the fruit.

The fruit fumigated with 1-MCP exhibited lowest (196.75 g kg^{-1}) mean sucrose levels, but highest (22.26 g kg^{-1}) mean sorbitol levels, compared to all other treatments (Table 4.5). The storage period or interaction between the storage period and ethylene antagonist treatments did not show any effect on mean values of sucrose and sorbitol.

4.3.2.5 Individual organic acids

The fruit samples studied using RP-HPLC system exhibited considerable amounts of malic acid and succinic acid, very low amounts of fumaric acid, tartaric acid and no citric acid. The fruit fumigated with BC and NC exhibited significantly higher levels of malic acid (6.27 g kg^{-1} and 5.93 g kg^{-1}, respectively) and succinic acid (0.64 g kg^{-1} and 0.63 g kg^{-1}, respectively) when compared to all other treatments (Table 4.6). The fruit fumigated with 1-MCP, control and ethanol dipped fruit showed least values of malic acid (3.32 g kg^{-1}, 3.15 g kg^{-1} and 3.58 g kg^{-1}, respectively) and succinic acid (0.39 g kg^{-1}, 0.38 g kg^{-1} and 0.38 g kg^{-1}, respectively) (Table 4.6). The was no significant effect of storage period nor significant interaction effect between the storage period and ethylene antagonist treatments on the levels of malic acid, succinic acid.

The levels of fumaric acid were significantly affected only by the storage period and the fruit cold-stored for 100 days (0.20 g kg^{-1}) exhibited significantly higher values than 150 days (0.17 g kg^{-1}) stored fruit (Table 4.6).

Table 4.4 Effect of the different formulations of ethylene antagonists on the levels of glucose (g kg^{-1}) and fructose (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in cold storage

Individual Sugars						
Storage period (Days)						
	Glucose			Fructose		
Treatment	100	150	Mean (T)	100	150	Mean (T)
Control	11.11±1.00	8.73±0.67	9.92C	160.25±6.33	163.28±2.33	161.77
Ethanol	7.18±0.42	7.81±0.58	7.49B	154.18±1.09	166.30±0.85	160.24
BC Fumigation	10.65±0.64	7.96±0.33	9.31C	162.33±4.16	164.01±3.23	163.17
BC Dip	7.38±0.35	5.09±0.55	6.24AB	157.36±3.63	161.36±1.51	159.36
NC Fumigation	9.14±0.40	5.46±0.60	7.30B	168.94±5.20	160.44±2.24	164.69
NC Dip	6.97±0.66	3.82±0.44	5.39A	166.94±0.54	157.78±3.64	162.36
1-MCP	9.70±1.01	8.70±0.34	9.20C	158.62±3.48	164.82±2.81	161.72
Mean (D)	8.88B	6.79A		161.23	162.57	
LSD ($P \leq 0.05$)	T=1.44	D=0.77	TXD=ns	T=ns	D=ns	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The upper case was used for treatment and storage period means.

Table 4.5 Effect of the different formulations of ethylene antagonists on the levels of sucrose (g kg^{-1}) and sorbitol (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in cold storage

Individual Sugars						
Storage period (Days)						
	Sucrose			Sorbitol		
Treatment	100	150	Mean (T)	100	150	Mean (T)
Control	198.17±5.93	204.47±2.27	201.32AB	17.93±0.73	19.01±0.42	18.47A
Ethanol	208.22±1.46	209.58±2.20	208.90BC	18.71±0.16	18.72±0.37	18.71AB
BC Fumigation	203.24±5.34	199.56±3.54	201.40AB	20.86±1.18	19.30±0.57	20.08BC
BC Dip	218.80±4.98	212.36±2.32	215.58CD	20.31±0.80	19.99±0.26	20.15BC
NC Fumigation	216.45±4.37	215.52±0.73	215.99CD	20.53±0.61	21.68±0.50	21.10CD
NC Dip	222.57±2.38	216.04±2.08	219.31D	21.92±0.29	21.40±0.70	21.66CD
1-MCP	200.00±3.44	193.50±2.01	196.75A	22.58±0.70	21.94±1.07	22.26D
Mean (D)	209.63	207.29		20.41	20.29	
LSD ($P \leq 0.05$)	T=7.54	D=ns	TXD=ns	T=1.46	D=ns	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The upper case was used for treatment and storage period means.

Table 4.6 Effect of the different formulations of ethylene antagonists on the levels of individual organic acids (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in cold storage

	Individual Acids								
	Storage period (Days)								
	Malic acid			Succinic acid			Fumaric acid		
Treatment	100	150	Mean (T)	100	150	Mean (T)	100	150	Mean (T)
Control	2.53±0.27	3.78±0.30	3.15A	0.42±0.03	0.33±0.02	0.38A	0.17±0.00	0.17±0.00	0.17
Ethanol	3.55±0.33	3.61±1.12	3.58A	0.38±0.05	0.38±0.05	0.38A	0.17±0.00	0.17±0.00	0.17
BC Fumigation	6.66±0.65	5.88±0.57	6.27C	0.66±0.07	0.63±0.07	0.64C	0.20±0.01	0.19±0.01	0.19
BC Dip	4.30±0.34	4.95±0.40	4.62AB	0.54±0.01	0.49±0.02	0.52BC	0.19±0.01	0.17±0.00	0.18
NC Fumigation	5.97±0.84	5.89±0.67	5.93C	0.66±0.08	0.60±0.06	0.63BC	0.19±0.02	0.19±0.01	0.19
NC Dip	5.51±0.43	5.64±0.24	5.58BC	0.53±0.12	0.47±0.09	0.50AB	0.21±0.01	0.17±0.00	0.19
1-MCP	3.93±0.91	2.72±0.83	3.32A	0.39±0.07	0.39±0.07	0.39A	0.25±0.05	0.17±0.00	0.21
Mean (D)	4.63	4.64		0.51	0.46		0.20B	0.17A	
LSD ($P \leq 0.05$)	T=1.39	D=ns	TXD=ns	T=0.13	D=ns	TXD=ns	T=ns	D=0.02	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The upper case was used for treatment and storage period means.

4.3.2.6 Total phenols, ascorbic acid and total antioxidant capacity

The Cripps Pink fruit treated with ethylene antagonists exhibited higher values of total phenols than control (26.15 g GAE kg^{-1}) and ethanol dipped fruit (27.79 g GAE kg^{-1}). The fruit fumigated with 1-MCP exhibited significantly highest (32.05 g GAE kg^{-1}) levels of total phenols (Table 4.7). The fruit cold-stored for 150 days (26.85 g GAE kg^{-1}) showed lower values of total phenols than fruit stored for 100 days (32.23 g GAE kg^{-1}) (Table 4.7). There was a significant interaction effect between the storage period and ethylene antagonists on the levels of total phenols. The control fruit cold-stored for 150 days (20.21 g GAE kg^{-1}) exhibited significantly lowest levels of total phenols when compared to all other treatments (Table 4.7).

There was no significant effect of the storage period or ethylene antagonist treatments on the ascorbic acid levels in the Cripps Pink fruit. The ethylene antagonist treatments did not show any significant effect even on the levels of total antioxidant activity. The Cripps Pink fruit cold-stored for 150 days (8.65 µM kg^{-1} Trolox) exhibited significantly lower total antioxidant capacity than 100 days stored fruit (9.60 µM kg^{-1} Trolox) (Table 4.7). A significant interaction effect between storage period and ethylene antagonist treatments was recorded and the control fruit cold-stored for 150 days exhibited significantly lowest (8.08 µM kg^{-1} Trolox) total antioxidant capacity compared to all other treatments (Table 4.7).

Table 4.7 Effect of the different formulations of ethylene antagonists on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of the Cripps Pink apple fruit stored in cold storage

	Storage period (days)		
Treatment	100	150	Mean (T)
	Total phenols (g GAE kg^{-1})		
Control	32.09±1.19cdefg	20.21±1.05a	26.15A
Ethanol	27.60±1.80bcd	27.98±2.33bcde	27.79AB
BC Fumigation	35.09±0.74fg	25.82±2.52b	30.45BC
BC Dip	36.58±1.13g	25.54±2.02b	31.06BC
NC Fumigation	33.31±2.27efg	27.04±0.50bc	30.17BC
NC Dip	27.88±1.48bcde	30.31±0.23bcdef	29.10ABC
1-MCP	33.03±0.36defg	31.06±1.51bcdefg	32.05C
Mean (D)	32.23B	26.85A	
LSD ($P \leq 0.05$)	T=3.48	D=1.86	TXD=4.92
	Ascorbic acid (g kg^{-1})		
Control	12.59±0.41	11.59±0.37	12.09
Ethanol	11.68±0.15	12.40±0.30	12.04
BC Fumigation	12.40±0.31	11.56±0.59	11.98
BC Dip	11.99±0.18	12.52±0.35	12.26
NC Fumigation	11.99±0.22	11.63±0.23	11.81
NC Dip	12.08±0.40	12.03±0.33	12.05
1-MCP	12.43±0.14	12.41±0.44	12.42
Mean (D)	12.17	12.02	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=ns
	Total antioxidant capacity (µM kg^{-1} Trolox)		
Control	9.81±0.21def	8.08±0.24a	8.95
Ethanol	9.41±0.07cde	8.56±0.23ab	8.98
BC Fumigation	10.24±0.31f	7.96±0.26a	9.10
BC Dip	10.17±0.20ef	8.95±0.21bc	9.56
NC Fumigation	9.65±0.17cdef	8.57±0.10ab	9.11
NC Dip	9.34±0.23bcd	9.44±0.09cde	9.39
1-MCP	8.60±0.13ab	9.02±0.30bcd	8.81
Mean (D)	9.60B	8.65A	
LSD ($P \leq 0.05$)	T=ns	D=0.27	TXD=0.71

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage period means.

4.4 Discussion

In the present study, the effects of fumigation and dip formulations of new ethylene antagonists (BC and NC) as well as 1-MCP fumigation on the postharvest fruit physiology and quality was investigated for the first time in Cripps Pink apple fruit. Irrespective of formulation the treatment with the new ethylene antagonists effectively reduced rates of ethylene production, respiration and delayed other changes related to ripening. These observations imply that the ethylene antagonists effectively blocked the ethylene receptors to hinder the action and retard autocatalytic production of ethylene in the fruit (Sisler, 2006). Ethylene gas is responsible for different physical, physiological and biochemical changes associated with ripening in fleshy fruit (Anwar et al., 2018). The effectiveness of fruit dip and fumigation in regulating ripening-related changes in the fruits were also compared. Overall, the fumigation treatment with BC, NC and 1-MCP were comparatively more effective than dip treatments, in significantly retarding the rates of the ethylene biosynthesis and maintaining other fruit quality parameters studied in the Cripps Pink fruit. The efficiency of the activity of ethylene antagonist compounds depends upon the application methods. The effective treatment concentration of the compound varies hugely with the difference in application method (Watkins, 2015).

The rates of ethylene climacteric peaks were reduced in Cripps Pink apple fruit treated with ethylene antagonists when compared to control or ethanol dipped fruit, irrespective of the formulation. This reduction could be ascribed to the capacity of 1-alkyl cyclopropenes to irreversibly attach with ethylene receptors to impede the action of internal as well as external ethylene in fruits (Sisler et al., 2003; Apelbaum et al., 2008). The mode of action of NC and BC in antagonising the ethylene actions is proposed to be similar to that 1-MCP (Musa, 2016). Compared to the fruit dipped in the BC and NC solutions, the fruit fumigated with ethylene antagonists (BC, NC and 1-MCP) exhibited reduced rates of ethylene and respiration climacteric peaks. Similarly, Argenta et al. (2007) found that 1-MCP dip treatment in Golden Delicious apples had equivalent ethylene antagonistic effect as 1-MCP fumigation only when 700 times of the concentration of the fumigation treatment was used to prepare the solution. The lower performance of BC and NC dip treatments when compared to the

respective fumigation treatments could be due to the less concentration of the treatments.

The Cripps Pink fruit fumigated with ethylene antagonists (BC, NC, 1-MCP) exhibited the lowest PLW values of all other treatments. The fruit dipped in ethylene antagonists showed lower PLW values when compared to control and ethanol dipped fruit. The reduction in fruit weight during storage is mainly due to loss of water from the fruit. The loss of water from the fruit is in turn due to postharvest physiological activities such as respiration and transpiration carried out by the fruit during storage (Becker and Fricke,1996). Reduction in rates of respiration and ethylene biosynthesis in the fruit lowers the PLW in the fruit during storage (Martínez-Romero et al., 2007). The mean PLW in fruit was higher in the fruit stored for 150 days when compared to 100 days. Longer the storage duration higher is the amount of respiration carried by the fruits, hence higher PLW in the fruit (Maguire et al., 2001).

The fruit firmness and crispness in apples are chief characteristics which decide consumer acceptability (DeEll et al., 2001). The firmness in the fruit fumigated with ethylene antagonists was significantly higher when compared to all other treatments. The hydrolysis of cell wall constituents and loss of cell turgor are important reasons for the reduction of flesh firmness in fruits during storage. The plant hormone ethylene plays an important role in the activation of cell wall hydrolysing enzymes (Giovannoni, 2008). The reduced rates of ethylene production in the fruit fumigated with ethylene antagonists could be the reason for the maintenance of higher fruit firmness (Giovannoni, 2008). The fruit cold-stored for 150 days exhibited comparatively lesser fruit firmness. The firmness of apple fruit reduces with the extension of storage period due to different continuous physiological activities which result in loss of cell turgor (DeEll et al., 2001).

The levels of TA were maintained high in the fruit fumigated with ethylene antagonists when compared to other dip treatments and control. Ethylene accelerates the ripening process which involves the breakdown of acids to meet the demand of respiratory substrates (Giovannoni, 2008). The high levels of TA in ethylene antagonist fumigated fruit could be related to effective inhibition of ethylene action (Fan et al. 1999). The variations in values of SSC and thereby SSC: TA ratio due to ethylene antagonist treatment did not follow any specific trend. This indicates that the SSC accumulation

in apple fruit during storage is not necessarily related to levels of ethylene perception of the fruit (Fan et al., 1999). Following 150 days cold storage the TA values were significantly lower, while SSC and SSC: TA values were higher when compared to fruit cold-stored for 100 days. These changes are due to break down of the acids to convert into simple sugars along the storage period. The simple sugars formed are used up as respiratory substrates with progress in fruit ripening (Giovannoni, 2008).

The changes in the levels of individual sugars due to ethylene antagonist treatments failed to follow a definite trend. These fluctuations in the sugar levels could be ascribed to different rates of interconversion among glucose, fructose, sucrose and sorbitol during the ripening process (Ackermann et al., 1992). The levels of individual sugars reduced from 100 days to 150 days of cold storage. The sugars are primary respiratory substrates for the respiration process and are used up in the respiration process during storage of fruit (Giovannoni, 2008). The malic acid and succinic acid were prominent acids in the Cripps Pink apple fruit. When compared to control and ethanol dip treatments, the levels of individual organic acids were higher in the fruit treated with ethylene antagonists. The maintenance of organic acid levels can be associated with reduced rates of ethylene production by the treatments with ethylene antagonists (Giovannoni, 2008).

The higher levels of total phenols were recorded in the fruit treated with ethylene antagonist when compared to control and ethanol dipped fruit. Fruit ripening process involves the production of several ROS and oxidative reactions. The bioactive phenolic compounds play a major role in degrading ROS formed and hence get depleted with the ripening process (Steinite et al., 2004; Valero et a., 2016). Hence higher levels of total phenols in fruit treated with ethylene antagonists could be attributed to delay in ripening process due to a reduction in ethylene production rates (Masia, 1998).

4.5 Conclusion

The rate of ethylene production and respiration were effectively reduced in the Cripps Pink apple fruit treated with the novel ethylene antagonists (BC and NC) irrespective of the formulations when compared to control and ethanol treatments, during 100 and 150 days of cold storage periods. The efficiency of fumigation treatments with BC and

NC were more effective in antagonising ethylene action when compared to the respective fruit dip treatments. The ethylene antagonist treatments retarded rates of ethylene production reduced PLW, maintained higher fruit firmness, TA and total phenol levels when compared to control and ethanol treatments. The efficiency of 1-MCP fumigation treatment in lowering the rates of ethylene and respiration climacteric peaks was significantly highest when compared to all other treatments. The effects of different concentrations of novel ethylene antagonist formulations on the rates of ethylene production and respiration as well as on the fruit quality of Cripps Pink apple warrant future investigation to perform as effective as 1-MCP.

CHAPTER 5

Effects of novel ethylene antagonists and ozone in regulating ethylene production and fruit quality of the cold-stored Cripps Pink and Granny Smith apples

Abstract

The postharvest quality of the fruit is determined directly or indirectly by the levels of internal ethylene produced and ethylene present in the storage environment. In the present experiment, effects of two potential ethylene antagonists namely 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC), as well as 1-methylcyclopropene (1-MCP) and ozone application on the ethylene production and postharvest quality of Cripps Pink and Granny Smith apple fruits cold-stored for 90 and 120 days, were investigated. The experiment fruit were fumigated with ethylene antagonists for 18 h in the 60 L hermetically sealable plastic drums and the untreated fruit were used as control. The experiment was designed in completely randomised block design and each treatment was replicated for four times with fifteen fruit per each experimental unit. The rates of ethylene and respiration climacteric peaks were significantly lowest in the Cripps Pink apple fruit fumigated with BC and NC whilst the Granny Smith apple fruit fumigated with 1-MCP showed lowest climacteric peak values. The Granny Smith and Cripps Pink fumigated with ethylene antagonists reduced PLW, maintained higher fruit firmness as well as higher levels of total phenols and total antioxidant capacity when compared to control fruit in 90 and 120 days of cold storage. The ozone application in cold storage aided in maintaining the postharvest fruit quality but has induced a rise in rates of ethylene production in both the apple cultivars studied following both 90 and 120 days of cold storage. There was no significant interaction effect between the ethylene antagonists and ozone application on the rates of ethylene production and respiration during the cold storage. The novel ethylene antagonists (BC and NC) possess the capacity to become an alternative to 1-MCP treatments.

5.1 Introduction

Based upon the trends of respiration rate during the ripening process, apple (*Malus* × *domestica* Borkh.) is categorised as climacteric fruit. They exhibit a sharp increase in respiration rate during ripening and this characteristic rise is termed as 'respiratory climacteric'. The occurrence of respiratory climacteric generally coincides with the initiation of fruit ripening process (Giovannoni, 2008). The fruit undergoes several interdependent physiological and biochemical events during the ripening process. Ripening process is generally considered as a final stage of the fruit development and is followed by senescence and deterioration. The sequence of events involved in the ripening process usually results in the breakdown of starch into sugars, degradation of cell wall materials, the formation of aroma volatiles, accumulation of pigment molecules and ultimately enhance aesthetic qualities of the fruit (Tucker, 2012).

Ethylene is a gaseous plant hormone produced in almost all the plant parts, generally as a response to different biotic and abiotic stresses (Grierson, 1987). It is involved in various plant physiological processes from seed germination to abscission of plant parts (Imaseki, 1991). The role of ethylene in promoting fruit ripening is one of the important functions. Galil (1968) enclosed the fig fruit in cellophane bags while on the trees, exposed it to ethylene gas and reported that exposure to external ethylene triggered fruit ripening. Apple fruit produces large amounts of ethylene during the ripening process and is also sensitive to external ethylene exposure. The magnitude of internal ethylene production and ethylene gas present in the storage environment, directly or indirectly affect storage life and postharvest quality of the fruit (Abeles et al., 1992).

Postharvest life of apple can be significantly extended by maintaining optimal storage conditions and efficient ethylene regulation (Gross et al., 2016). Storing the fruit in low temperatures can increase the storage life by lowering rates of respiration and ethylene biosynthesis (Gross et al., 2016). The enzyme activity in the fruits is significantly lowered when stored at low temperatures and hence reduce fruit softening, weight loss, chlorophyll degradation and other ripening-related physiological changes (Leja et al., 2003). There are different methods available to counteract the negative effects of ethylene in the fruits. The cyclopropenes (CP) are effective ethylene antagonists and 1-MCP is recognised as relatively stable CP at

gaseous state. The 1-MCP is commercially being used in the horticulture industry around the world (Valero et al., 2016).

Ozone is a powerful oxidising agent and well known for its biocidal properties (Öztekin, 2018). It has been used in the horticulture industry to sanitise the water before using it for pre-cooling of the fruit. Washing the fruit with ozonated water eliminates any existing microbial population on the fruit surface and prevent further multiplication of microbes (Tzortzakis and Chrysargyris, 2017). The fruit treated with ozone does not show any residues as the ozone molecule readily dissociates into oxygen gas (Selma et al., 2008). Ozone also oxidises the ethylene gas in the storage environment but showed little effect on the levels of internal ethylene production in the fruit (Skog and Chu, 2001). The fruit treated with ozone retained firmness, aroma volatiles and exhibited increased levels of sugars and anthocyanins during storage (Tokala et al., 2018). The postharvest quality of fruits is significantly affected by ozone treatment, but the positive or negative effects depends upon the concentration of ozone, fruit type and storage temperature. The fruits treated with ozone showed a relative increase in the storage life due to reduced microbial load, the breakdown of ethylene and consequently by a reduction in fruit deterioration. The efficacy of ozone treatment in maintaining fruit quality and extension of storage life was higher when applied in combination with cold storage, CA storage or with 1-MCP (Tokala et al., 2018).

Although the positive effects of 1-MCP are popular in the horticulture industry, they still have some limitations. The 1-MCP is stable only at the gaseous state and commercially it is still available as service rather than as a compound. Hence, there is a need to explore possibilities to develop compounds as effective as 1-MCP and easy to handle. There are very few studies that report the combined effects of ozone and ethylene antagonists on the rate of ethylene production, respiration and other postharvest quality parameters in cold-stored fruits. Keeping these points in view, in the following experiments the combined effects of two newly developed ethylene antagonists (BC and NC) and ozone on ethylene production, respiration and fruit quality parameters during two different cold storage durations were investigated in Cripps Pink and Granny Smith apple fruit for the first time. It was hypothesised that the novel ethylene antagonists will effectively antagonise ethylene action and maintain

fruit quality during cold storage. It was also hypothesised that the combination of ethylene antagonist treatments and ozone in cold storage would synergistically contribute to retard ethylene production and maintain consumer preferable fruit quality in both the apple cultivars studied.

5.2 Material and methods

5.2.1 Fruit and experimental conditions

Two separate experiments were conducted with Cripps Pink and Granny Smith apple fruit to study the effects of the novel ethylene antagonists and ozone on fruit physiological and quality parameters during cold storage.

5.2.1.1 Experiment 1: Effects of novel ethylene antagonists and ozone on postharvest physiology and quality of cold-stored Cripps Pink apple fruit

Cripps Pink apple fruit were sourced from Manjimup (34°13' S latitude, 116°08' E longitude) at the commercial mature stage (fruit firmness 65.95 ± 3.49 N; SSC 13.7 ± 0.08 %; TA 0.74 ± 0.02 %) during 9th May 2017. The fruit were collected from 11-year old trees grafted on M26 rootstock and planted in North-South direction with a spacing of 4.5 m (within rows) and 1 m (between rows). The trees were trained in modified central leader system. After the harvest the fruit were dipped in an aqueous solution mixture of 'Scholar' (a.i. 230 g L^{-1} fludioxonil) @ 2.00 mL L^{-1}, 'Caltop' (a.i. 165 g L^{-1} calcium) @ 7.00 mL L^{-1}, 'DPA' (diphenylamine) @ 1.70 mL L^{-1} to prevent postharvest diseases and disorders during storage. The fruit were then placed in soft board trays, packed in corrugated cardboard boxes and immediately transported to the Curtin Horticulture Research Laboratory, Perth in the air-conditioned vehicle.

The fruits with uniform size, free from any mechanical injuries, bruises and diseases were selected for the experiment. The fruit were treated with the ethylene antagonists by fumigation as explained in Chapter 3, Section 3.3.1, keeping sixty fruits per drum. The untreated fruit were considered as control. After the fumigation treatment, the fruits were packed in the corrugated cardboard boxes with softboard trays with respect to the treatment. The experiment was laid in completely randomised block design and all the treatments were replicated four times with fifteen fruit per replication. Sixty

fruit (fifteen fruit replicated four times) of respective treatment were arranged in one box. All the boxes were then divided into two lots, one lot meant for storage in cold storage (0 ± 2 °C) with ozone and another lot for cold storage (0 ± 2 °C) without ozone (details mentioned in Chapter 3, Section 3.4.3) at grower's property at Manjimup. The ozone generators continuously produced ozone gas (0.1 ± 0.08 µL L-1) and the ozone gas concentration was monitored using 'Aeroqual™' series 500 monitor fitted with Aeroqual IP41 Remote Sensor Kit (Aeroqual Ltd., Auckland, New Zealand) (Figure 3.4A, B). Each lot was again divided into two sublots, one meant to be stored for 90 days and another for 120 days storage. The storage duration was scheduled according to the regular storage practices of the local apple grower. The fruit were removed from the storage on completion of designated storage period and physiological and quality parameters of the fruit were then analysed.

5.2.1.2 Experiment 2: Effects of novel ethylene antagonists and ozone on postharvest physiology and quality of cold-stored Granny Smith apple fruit

Granny Smith apple fruit at the commercial mature stage (fruit firmness 55.06 ± 3.21 N; SSC 10.94 ± 0.05 %; TA 0.61 ± 0.03 %) were sourced from Manjimup (34°13' S latitude, 116°08' E longitude) in 21st April 2017. The fruit were harvested from 11-year old Granny Smith trees grafted on M26 rootstock. The trees were trained in modified central leader system and planted in North-South direction with a spacing of 4.5 m (within rows) and 1 m (between rows). After the harvest, the fruit were treated and handled similarly to Experiment 1 (Section 5.2.1.1).

Both the experiments were laid following completely randomised design (CRD) with fifteen fruits as each experimental unit and four replicates.

5.2.2 Determination of fruit physiological parameters

5.2.2.1 Rate of ethylene production

The ethylene production rate in apple fruit was estimated using two fruits per experimental unit enclosed in an air-tight one-litre glass jar. A sample (one mL) of the headspace gas from the individual glass jars, sealed for 1 h (at 20 ± 2 °C), was injected into GC (6890N Network GC system, Agilent Technology, CA, USA) to determine

the amount of ethylene produced. The ethylene production rate was estimated daily until the climacteric peak was achieved. The detailed procedure and calculations for ethylene estimation are explained in Chapter 3, Section 3.5.1. The rate of ethylene production is expressed as μmol C_2H_4 kg^{-1} h^{-1}.

5.2.2.2 Rate of respiration

Two fruits per experimental unit were used to estimate the respiration rate in apple fruit during ripening. The two mL gas sample drawn from the headspace of the one-litre glass jars, sealed for one hr (at 20 ± 2 °C), was injected into Infrared gas analyser (Servomex Gas Analyser, 1450 Food Package Analyser, Servomex Limited, UK) to estimate respiration rate based upon the carbon dioxide evolved. The procedure and calculations to estimate respiration are explained in detail in Chapter 3, Section 3.5.2. The respiration rate in apple fruit is expressed as mmol CO_2 kg^{-1} h^{-1}.

5.2.3 Determination of fruit quality parameters

5.2.3.1 Physiological loss of weight (PLW)

Fifteen fruits per experimental unit were weighed just before shifting into designated storage rooms and then weighed again after completion of the storage period. The PLW was then calculated by using the formula as explained in Chapter 3, Section 3.6.1. The PLW of apple fruit is expressed as %.

5.2.3.2 Fruit firmness

Ten fruits per experimental unit were randomly chosen to determine the fruit firmness, on completion of the designated storage period. Texture Analyser (TA Plus, Ametek Lloyd Instruments Limited, UK) was used to measure fruit firmness by following the procedure explained in Chapter 3, Section 3.6.2. The fruit firmness is expressed as newtons (N).

5.2.3.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA

The juice extracted from slices taken from thirteen fruits in each replicate were pooled and the samples were used to estimate SSC, TA and SSC: TA following the procedures

mentioned in Chapter 3, Section 3.6.3. The SSC is expressed as % Brix and TA as % malic acid.

5.2.3.4 Individual sugars and organic acids

The quantities of individual sugars (glucose, fructose, sucrose and sorbitol) and organic acids (malic acid, succinic acid, fumaric acid, tartaric acid and citric acid) were estimated using reverse-phase HPLC system (Waters 1525, Milford Corporation, USA). Dual λ UV absorbance detector (Water 2487, Milford Corporation, USA) at 214 nm was used to determine individual organic acids, while the individual sugars were detected by Refractive Index (RI) detector (Water 2414, Milford Corporation, USA). The detail description of instruments and the determination procedure are mentioned in Chapter 3, Section 3.6.5. The estimated levels of sugars and organic acids are expressed as g kg^{-1}.

5.2.3.5 Total phenols

The levels of total phenols in the fruit pulp samples were estimated following the method outlined by Vithana et al. (2018) with a few modifications in the procedure described by Robles-Sánchez et al. (2009). The procedure for sampling and total phenol estimation using Folin-Ciocalteu reagent is detailed in Chapter 3, Section 3.6.6. The total phenolic content was calculated relating to the standard curve of pure gallic acid and expressed as g gallic acid equivalents (GAE) kg^{-1} fresh weight basis.

5.2.3.6 Ascorbic acid

The ascorbic acid levels in the fruit pulp were determined using UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK) following the procedure as outlined in Chapter 3, Section 3.6.7. The levels of ascorbic acid were calculated with respect to the standard L-ascorbic acid curve and expressed as g kg^{-1} fresh weight basis.

5.2.3.7 Total antioxidant capacity

The total antioxidant capacity in the fruit pulp was determined using DPPH method following the procedure described in Chapter 3, Section 3.6.8. The levels of total

antioxidant capacity were estimated based upon the Trolox standard curve and expressed as µM kg^{-1} Trolox fresh weight basis.

5.2.4 Statistical analysis

The data recorded was statistically analysed using GenStat software version 14.0 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The results were presented as means ± standard errors (SE) of the mean. The data were analysed by two-way analysis of variance (ANOVA) with ethylene antagonist treatment and storage type as two factors. The least significant difference (LSD) was determined following F-test with 5 % error probability. Duncan multiple comparison tests were used to comparing the treatment means.

5.3 Results

5.3.1 Fruit physiological parameters

5.3.1.1 Ethylene (C_2H_4) production

The onset of ethylene climacteric peak was significantly ($P \leq 0.05$) delayed with the fumigation of BC, NC and 1-MCP in 90 and 120 days cold-stored Cripps Pink and Granny Smith apple fruit (Table 5.1 and 5.2, Figure 5.1 A-D). The mean ethylene climacteric peak onset was not significantly affected by the presence or absence of the ozone gas in both 90 and 120 days of cold storage in Cripps Pink apples. In Granny Smith, the fruit stored in ozonised cold store for 90 days showed the significantly early (7.12 days) onset of ethylene climacteric peak. The interaction between the ethylene antagonist fumigation treatments and ozonised cold storage did not significantly affect the onset of ethylene climacteric peak in 90 and 120 days cold-stored Cripps Pink apples and in Granny Smith apples stored for 120 days.

The mean ethylene climacteric peak rates were significantly reduced with the fumigation treatments when compared to control in the cold-stored Cripps Pink and Granny Smith fruit (Figure 5.1). The significantly lowest ethylene climacteric peak rates were observed in the Cripps Pink fruit fumigated with BC and NC stored for 90 (0.26 and 0.18 µmol $kg^{-1}h^{-1}$ respectively) and 120 days (0.18 and 0.16 µmol $kg^{-1}h^{-1}$ respectively) (Table 5.1). The ethylene climacteric peak rates in Granny Smith fruit

were significantly lowest in the fruit fumigated with 1-MCP (0.02 µmol $kg^{-1}h^{-1}$) followed by NC (0.76 µmol $kg^{-1}h^{-1}$) and then BC treated fruit (1.89 µmol $kg^{-1}h^{-1}$) (Table 5.2). The fruit stored for 90 and 120 days in the cold storage with ozone showed higher mean ethylene peak rates than those stored without ozone gas in both Cripps Pink and Granny Smith apples (Table 5.1 and 5.2). There was no significant interaction effect between ethylene antagonist treatments and the cold storage type on the ethylene climacteric peak rates in both Cripps Pink and Granny Smith apple fruit stored for 90 and 120 days.

5.3.1.2 Respiration (CO_2 production)

The fruit fumigated with BC, NC and 1-MCP exhibited the delayed onset of the respiratory climacteric peak over control in both Cripps Pink and Granny Smith apple fruit (Table 5.1 and 5.2). The presence or absence of ozone gas in the cold storage did not show a significant effect on the mean respiratory climacteric peak onset in Cripps Pink fruit cold-stored for 90 days and in Granny Smith fruit stored for 90 and 120 days (Table 5.1 and 5.2). The interaction between cold storage type and ethylene antagonist treatment on the mean respiratory climacteric peak onset was non-significant in the Granny Smith fruit cold-stored for 90 and 120 days and in Cripps Pink fruit cold-stored for 90 days (Table 5.1 and 5.2).

The mean respiratory climacteric peak rates were comparatively lower in the fruit fumigated with BC, NC and 1-MCP than in control fruit for both Cripps Pink and Granny Smith (Table 5.1 and 5.2). The mean rates of the respiratory climacteric peak were significantly higher in the Cripps Pink apple cold-stored for 90 days with ozone gas when compared to non-ozonised, but then there was no significant effect in fruit cold-stored for 120 days (Table 5.1). The presence of ozone gas did not show a significant effect on the mean respiratory climacteric peak rate in the Granny Smith fruit stored for 90 and 120 days (Table 5.2). The interaction effect between ethylene antagonist treatments and type of cold storage for mean rates of the respiratory climacteric peak was not-significant in both Cripps Pink and Granny Smith fruit cold-stored for 90 and 120 days (Table 5.1 and 5.2).

Table 5.1 Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0 ± 2 °C) with or without ozone gas on the climacteric peak onset (days) and peak rate of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) of the Cripps Pink apple fruit

		Storage period (days)					
		90			120		
Parameters	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
C_2H_4 climacteric peak onset	Control	8.00±0.00	10.75±0.25	9.38A	7.25±1.25	9.75±0.25	8.50A
	BC	17.25±0.25	17.75±1.25	17.50BC	12.50±1.44	13.25±0.63	12.88B
	NC	17.50±1.19	15.25±2.43	16.38B	12.75±1.25	13.50±1.19	13.12B
	1-MCP	19.00±0.00	18.50±0.29	18.75C	14.00±0.41	10.25±1.03	12.12B
	Mean (S)	15.44	15.56		11.62	11.69	
	LSD ($P \leq 0.05$)	T=1.92	S=ns	TXS=ns	T=2.46	S=ns	TXS=ns
C_2H_4 climacteric peak rate	Control	1.27±0.10	1.81±0.15	1.54C	1.38±0.13	1.94±0.23	1.66C
	BC	0.22±0.08	0.30±0.09	0.26A	0.11±0.06	0.26±0.05	0.18A
	NC	0.12±0.02	0.23±0.11	0.18A	0.20±0.05	0.11±0.04	0.16A
	1-MCP	0.81±0.09	1.03±0.07	0.92B	0.50±0.08	0.49±0.12	0.49B
	Mean (S)	0.60A	0.84B		0.55	0.70	
	LSD ($P \leq 0.05$)	T=0.19	S=0.14	TXS=ns	T=0.25	S=ns	TXS=ns
Respiration climacteric peak onset	Control	6.00±1.68	5.00±0.00	5.50A	10.50±0.29bc	4.00±0.00a	7.25A
	BC	11.50±3.23	11.50±0.50	11.50B	8.50±0.29b	11.00±0.41bc	9.75B
	NC	11.25±3.09	11.25±0.25	11.25B	11.50±1.19bc	9.25±1.80bc	10.38B
	1-MCP	9.75±0.25	11.25±0.63	10.50B	11.75±0.48c	10.50±1.50bc	11.12B
	Mean (S)	9.62	9.75		10.56B	8.69A	
	LSD ($P \leq 0.05$)	T=3.29	S=ns	TXS=ns	T=2.02	S=1.43	TXS=2.86
Respiration climacteric peak rate	Control	0.95±0.08	1.06±0.09	1.01	1.07±0.08	0.98±0.05	1.02B
	BC	0.77±0.03	0.96±0.09	0.86	0.78±0.08	0.81±0.09	0.80A
	NC	0.76±0.15	0.85±0.14	0.81	0.76±0.14	0.67±0.08	0.71A
	1-MCP	0.79±0.09	1.13±0.18	0.96	0.84±0.04	0.89±0.07	0.87AB
	Mean (S)	0.82A	1.00B		0.86	0.84	
	LSD ($P \leq 0.05$)	T=ns	S=0.18	TXS=ns	T=0.16	S=ns	TXS=ns

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (2 fruit per replication), mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range test at ($P \leq 0.05$). Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage means. 90 and 120 days data were analysed separately.

Table 5.2 The climacteric peak onset (days) and peak rates of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) influenced by the ethylene antagonists and ozone in the Granny Smith apple fruit stored in cold storage (0 ± 2 °C) for 90 and 120 days.

		Storage period (days)					
		90			120		
Parameters	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
C_2H_4 climacteric peak onset	Control	3.50±0.29a	4.50±0.96a	4.00A	4.00±1.35	8.00±0.00	6.00A
	BC	10.00±0.00c	5.00±0.41a	7.50B	8.00±0.00	8.00±0.00	8.00B
	NC	11.00±0.41cd	7.75±1.31b	9.38C	8.75±0.75	9.75±0.25	9.25B
	1-MCP	12.00±0.00d	11.25±0.25cd	11.62D	10.25±1.11	11.50±0.29	10.88C
	Mean (S)	9.12B	7.12A		7.75	9.31	
	LSD ($P \leq 0.05$)	T=1.29	S=0.91	TXS=1.82	T=1.42	S=ns	TXS=ns
C_2H_4 climacteric peak rate	Control	2.57±0.23	2.51±0.32	2.54D	2.80±0.22	2.97±0.19	2.89D
	BC	1.71±0.03	2.06±0.17	1.89C	2.28±0.03	2.71±0.14	2.49C
	NC	0.32±0.12	1.20±0.16	0.76B	0.75±0.11	0.84±0.12	0.80B
	1-MCP	0.00±0.00	0.04±0.02	0.02A	0.02±0.01	0.05±0.02	0.04A
	Mean (S)	1.15A	1.45B		1.46	1.64	
	LSD ($P \leq 0.05$)	T=0.36	S=0.25	TXS=ns	T=0.28	S=ns	TXS=ns
Respiration climacteric peak onset	Control	3.75±0.63	4.75±1.11	4.25A	7.00±2.04	8.00±0.00	7.50
	BC	8.75±0.48	7.25±2.17	8.00B	7.50±1.19	8.25±0.25	7.88
	NC	9.00±1.00	9.00±1.15	9.00B	8.00±1.00	8.00±0.00	8.00
	1-MCP	6.00±1.78	7.25±1.89	6.62AB	10.25±0.25	9.75±0.48	10.00
	Mean (S)	6.88	7.06		8.19	8.50	
	LSD ($P \leq 0.05$)	T=3.02	S=ns	TXS=ns	T=ns	S=ns	TXS=ns
Respiration climacteric peak rate	Control	1.11±0.13	1.21±0.23	1.16	1.11±0.06	0.97±0.08	1.04C
	BC	0.95±0.09	0.93±0.04	0.94	0.91±0.07	0.87±0.06	0.89B
	NC	1.06±0.26	1.06±0.04	1.06	0.78±0.05	0.74±0.04	0.76A
	1-MCP	0.80±0.10	0.83±0.10	0.82	0.75±0.04	0.63±0.05	0.69A
	Mean (S)	0.98	1.01		0.89	0.80	
	LSD ($P \leq 0.05$)	T=ns	S=ns	TXS=ns	T=0.12	S=ns	TXS=ns

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (2 fruit per replication), mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range test at ($P \leq 0.05$). Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage means. 90 and 120 days data were analysed separately.

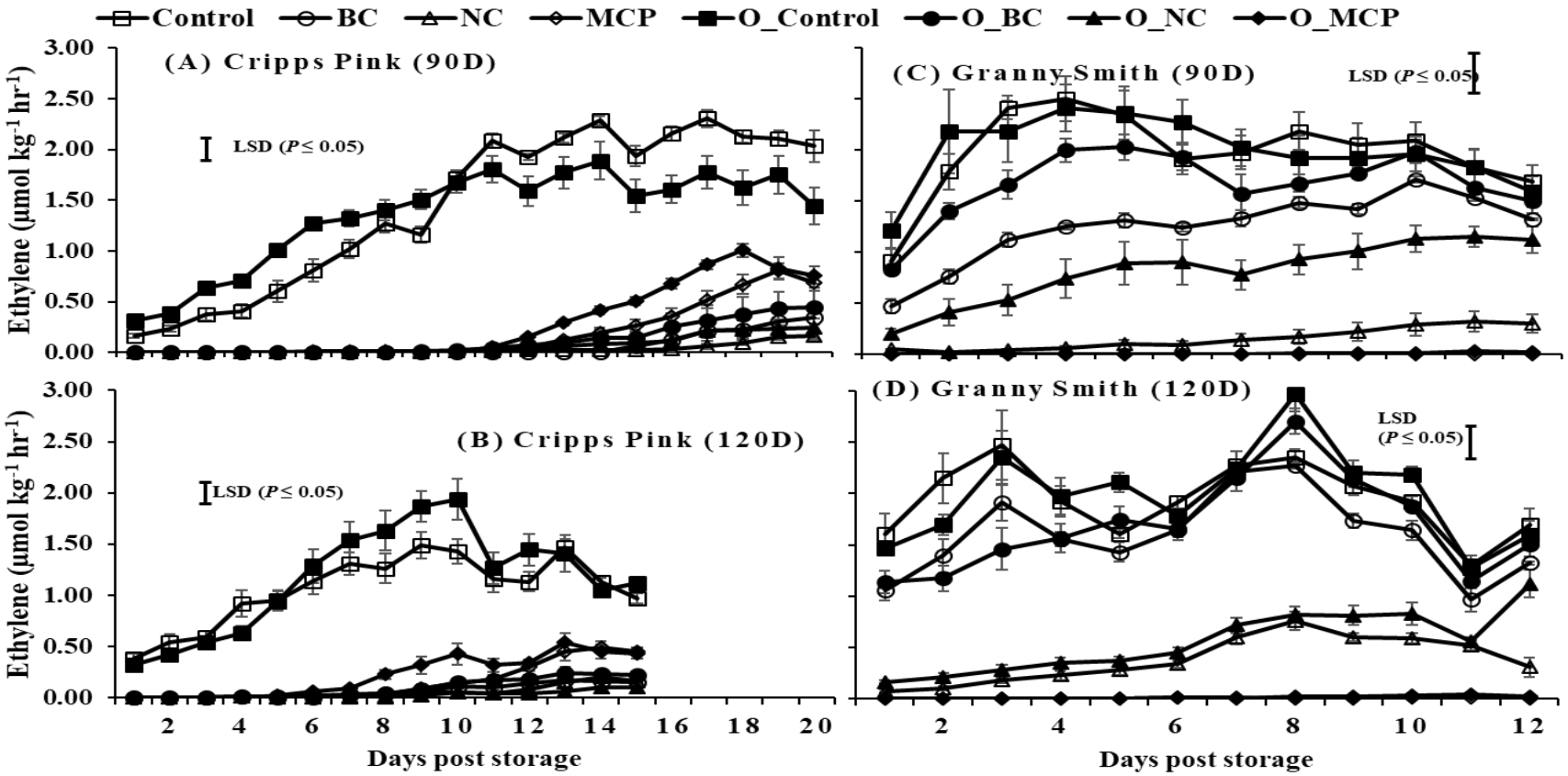


Figure 5.1 Changes in the rate of ethylene production due to ozone and ethylene antagonist treatment (T) days post storage (D) in Cripps Pink and Granny Smith apple fruits cold-stored for 90 and 120 days. Vertical bars represent SE of mean values and are not visible when values are smaller than the symbol. n=4 replicates (2 fruit per replication). LSD ($P \leq 0.05$) (A) T=0.05, D=0.08, TXD=0.21 (B) T=0.06, D=0.08, TXD=0.21 (C) T=0.11, D=0.14, TXD=0.39 (D) T=0.09, D=0.11, TXD=0.31

5.3.2 Fruit quality parameters

5.3.2.1 Physiological loss of weight (PLW) (%)

The Cripps Pink and Granny Smith fruit fumigated with BC, NC and 1-MCP, cold-stored (0±1°C) for 90 and 120 days with or without ozone exhibited lower mean PLW as compared to control. The mean PLW was significantly lower in the Cripps Pink apple fruit in ozonised cold storage (1.24 and 1.58%) than those stored without ozone (1.60 and 2.19%) for both 90 and 120 days, respectively (Table 5.3). In Granny Smith fruit, the presence or absence of ozone gas did not show any significant effect on the PLW for both 90 and 120 days of cold storage (Table 5.4) The interaction effect between the different treatments and cold storage types with and without ozone on PLW of Cripps Pink were found to be non-significant in both 90 and 120 days of storage (Table 5.3). The interaction effect between the different treatments and ozonised or non-ozonised cold storage types was significant in 90 days stored Granny Smith apple. The fruit fumigated with 1-MCP and stored for 90 days in ozonised cold storage exhibited lowest PLW (0.45%) when compared to all other treatments (Table 5.3). The interaction effect between cold storage type and ethylene antagonist treatment on PLW was non-significant in 90 and 120 days stored Cripps Pink fruit and in Granny Smith fruit cold-stored for 120 days (Table 5.3 and 5.4)

5.3.2.2 Fruit firmness (N)

The Granny Smith fruit fumigated with BC, NC and 1-MCP exhibited significantly higher firmness when compared to control fruit in both 90 and 120 days cold-stored fruit. The 1-MCP treated Granny Smith fruit had the highest firmness (48.01N and 48.91N) for 90 and 120 days, respectively followed BC and NC treated fruit (Table 5.4). Similarly, the BC, NC and 1-MCP fumigated Cripps Pink fruit also had higher firmness, but the difference was significant only in 120-day cold-stored fruit (Table 5.3). The ozonised or non-ozonised cold storage did not significantly affect the fruit firmness in 90 and 120 days stored Cripps Pink, and 120 days stored Granny Smith fruit. The Granny Smith fruit cold-stored for 90 days in the presence of ozone gas exhibited significantly lower firmness (43.74N) than one without ozone (45.26N) (Table 5.4). The interaction effect between ethylene antagonist treatments

Table 5.3 Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0 ± 2 °C) with or without ozone gas on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Cripps Pink apple fruit

		Storage period (days)					
		90			120		
Parameters	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
Physiological loss of weight (PLW)	Control	1.89±0.22	1.64±0.07	1.77	2.39±0.17	1.73±0.17	2.06
	BC	1.54±0.28	0.85±0.03	1.19	2.21±0.06	1.61±0.04	1.91
	NC	1.56±0.28	1.31±0.17	1.43	2.03±0.16	1.55±0.02	1.79
	1-MCP	1.43±0.19	1.17±0.17	1.30	2.13±0.18	1.42±0.17	1.78
	Mean (S)	1.60B	1.24A		2.19B	1.58A	
	LSD ($P \leq 0.05$)	T=ns	S=0.32	TXS=ns	T=ns	S=0.23	TXS=ns
Fruit firmness	Control	56.86±1.91	56.26±0.63	56.56	50.64±0.48	49.61±0.78	50.12A
	BC	57.32±0.57	57.41±0.79	57.37	54.09±1.40	53.89±0.88	53.99B
	NC	58.31±1.22	58.23±2.30	58.27	53.67±0.43	52.47±0.66	53.07B
	1-MCP	57.95±0.44	57.46±2.26	57.70	52.91±0.63	54.10±1.28	53.51B
	Mean (S)	57.61	57.34		52.83	52.52	
	LSD ($P \leq 0.05$)	T= ns	S=ns	TXS=ns	T=1.77	S=ns	TXS=ns

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (15 fruit (PLW) and 10 fruit (fruit firmness) per replication), Mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range test at ($P \leq 0.05$). Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The upper case was used for treatment and storage means. 90 and 120 days data were analysed separately.

Table 5.4 The physiological loss of weight (PLW) (%) and fruit firmness (N) influenced by the ethylene antagonists in the Granny Smith apple fruit stored in cold storage (0 ± 2 °C) for 90 and 120 days.

		Storage period (days)					
		90			120		
Parameters	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
Physiological loss of weight (PLW)	Control	1.16±0.18bc	1.01±0.11abc	1.09	1.54±0.18	1.44±0.13	1.49
	BC	0.62±0.01ab	1.08±0.13bc	0.85	1.21±0.22	1.56±0.17	1.38
	NC	1.31±0.24c	0.72±0.09c	1.02	1.53±0.15	1.30±0.31	1.28
	1-MCP	0.77±0.25abc	0.45±0.13a	0.61	1.24±0.01	1.24±0.22	1.24
	Mean (S)	0.97	0.81		1.38	1.32	
	LSD ($P \leq 0.05$)	T=ns	S=ns	TXS=0.53	T=ns	S=ns	TXS=ns
Fruit firmness	Control	40.70±0.56	38.77±0.69	39.73A	38.70±1.41	37.85±1.00	38.28A
	BC	46.49±1.03	45.40±0.70	45.95C	40.48±0.65	42.81±0.75	41.65B
	NC	44.77±0.56	43.84±0.64	44.31B	44.61±0.40	44.61±0.43	44.61C
	1-MCP	49.08±0.75	46.94±0.36	48.01D	49.63±0.32	48.20±0.47	48.91D
	Mean (S)	45.26B	43.74A		43.35	43.37	
	LSD ($P \leq 0.05$)	T= 1.58	S=0.54	TXS=ns	T= 1.77	S=ns	TXS=ns

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (15 fruit (PLW) and 10 fruit (Firmness) per replication), Mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range test at ($P \leq 0.05$). Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage means. Mean values without letters within columns or rows are non-significant. 90 and 120 days data were analysed separately.

and type of cold storage on fruit firmness was not-significant in both Cripps Pink and Granny Smith fruit cold-stored for 90 and 120 days (Table 5.3 and 5.4).

5.3.2.3 Soluble solid concentration (SSC), titratable acidity (TA) and SSC: TA

The Cripps Pink fruit fumigated with ethylene antagonists showed a significant decrease in mean SSC values, whilst the Granny Smith fruit fumigated with 1-MCP exhibited significantly highest mean SSC values for both 90 and 120 days of cold storage (Table 5.5 and 5.6). The cold storage type had a significant effect on the mean SSC, with fruit in ozonised cold storage having higher values for 90 days but had lower SSC values following 120 days when compared to non-ozonised cold storage in both Cripps Pink and Granny Smith fruit. There was a significant interaction effect between ethylene antagonist treatments and cold storage type on the SSC values in 90 and 120 days stored Cripps Pink fruit and in Granny Smith fruit cold-stored for 120 days (Table 5.5 and 5.6).

The BC, NC and 1-MCP fumigation did not show any significant effect on the TA value of Cripps Pink and Granny Smith cold-stored for 90 and 120 days (Table 5.5 and 5.6). There was no significant effect of ozonised cold storage on the TA values of 90 days stored Cripps Pink and Granny Smith fruit. The Cripps Pink fruit stored in ozonised cold storage for 120 days showed significantly lower TA values, whilst significantly higher TA values were exhibited by the 120 days cold-stored Granny Smith fruit with ozone (Table 5.5 and 5.6). The interaction between cold storage type and ethylene antagonist treatment was significant for the TA values in the Cripps Pink and Granny Smith fruit cold-stored for 120 days.

The ethylene antagonist fumigation, ozone treatment and the interaction between them showed no significant effect on the SSC: TA values in the Cripps Pink fruit cold-stored for 90 days (Table 5.5). The Granny Smith fruit fumigated with BC cold-stored for 90 days showed significantly highest SSC: TA (Table 5.6). The Cripps Pink fruit stored in ozonised cold storage for 120 days showed significantly higher SSC: TA values, whilst significantly lower TA values were exhibited by the 120 days cold-stored Granny Smith fruit with ozone (Table 5.5 and 5.6). The interaction between cold storage type and ethylene antagonist treatment significantly affected SSC: TA values in the Cripps Pink fruit stored for 120 days and fruits fumigated

Table 5.5 Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0 ± 2°C) with or without ozone gas on the changes in the soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Cripps Pink apple fruit

		Storage period (Days)					
		90			120		
Parameters	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
Soluble solids concentration (SSC)	Control	14.23±0.04a	15.92±0.14d	15.08B	14.33±0.02b	14.78±0.02d	14.55C
	BC	14.83±0.06b	15.08±0.17bc	14.95B	14.98±0.02e	14.18±0.02a	14.58C
	NC	14.20±0.07a	15.18±0.04c	14.69A	14.60±0.04c	14.33±0.04b	14.46B
	1-MCP	14.40±0.07a	14.75±0.06b	14.58A	14.58±0.02c	14.18±0.04a	14.38A
	Mean (S)	14.41A	15.23B		14.62B	14.36A	
	LSD ($P \leq 0.05$)	T=0.22	S=0.16	TXS=0.31	T=0.07	Str=0.05	TXS=0.10
Titratable acidity (TA)	Control	0.58±0.01	0.60±0.01	0.59	0.51±0.01a	0.55±0.01b	0.53
	BC	0.59±0.02	0.59±0.02	0.59	0.58±0.01bc	0.55±0.02b	0.57
	NC	0.55±0.01	0.58±0.01	0.57	0.57±0.02bc	0.50±0.01a	0.54
	1-MCP	0.58±0.02	0.61±0.02	0.60	0.59±0.01c	0.49±0.01a	0.54
	Mean (S)	0.58	0.60		0.57B	0.53A	
	LSD ($P \leq 0.05$)	T=ns	S=ns	TXS=ns	T=ns	S=0.02	TXS=0.35
SSC: TA	Control	24.43±0.36	26.44±0.39	25.43	27.98±0.48cd	26.76±0.45bc	27.37
	BC	25.02±0.43	25.52±0.91	25.27	25.72±0.42ab	25.74±0.76ab	25.73
	NC	25.72±0.44	26.07±0.48	25.89	25.57±0.71ab	28.55±0.55cd	27.06
	1-MCP	24.73±0.44	24.12±0.61	24.43	24.60±0.39a	28.82±0.54d	26.71
	Mean (S)	24.98	25.54		25.97A	27.47B	
	LSD ($P \leq 0.05$)	T=ns	S=ns	TXS=ns	T = ns	S = 0.88	TXS=1.75

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), Mean ± SE. The Duncan's multiple range test at ($P \leq 0.05$) was used to test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage means. 90 and 120 days data were analysed separately.

Table 5.6 The soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA influenced by the ethylene antagonists in the juice of Granny Smith apple fruit stored in cold storage (0 ± 2 °C) for 90 and 120 days.

		Storage period (days)					
		90			120		
Parameters	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
Soluble solids concentration (SSC)	Control	11.65±0.52	11.78±0.34	11.71A	12.53±0.06c	12.13±0.04b	12.32AB
	BC	12.10±0.12	11.90±0.41	12.00AB	12.75±0.08d	11.78±0.07a	12.26A
	NC	11.85±0.44	12.68±0.22	12.26B	12.40±0.09c	12.50±0.06c	12.45B
	1-MCP	12.50±0.33	13.12±0.16	12.81C	13.15±0.02e	12.15±0.02b	12.65C
	Mean (S)	12.03A	12.37B		12.70B	12.14A	
	LSD ($P \leq 0.05$)	T=0.41	S=0.30	TXS=ns	T=0.15	S=0.10	TXS=0.21
Titratable acidity (TA)	Control	0.59±0.01	0.63±0.03	0.61	0.50±0.01a	0.56±0.01e	0.53
	BC	0.61±0.02	0.59±0.02	0.60	0.52±0.0abcd	0.51±0.01abc	0.52
	NC	0.59±0.01	0.55±0.02	0.57	0.51±0.01ab	0.55±0.01bde	0.53
	1-MCP	0.59±0.01	0.65±0.01	0.62	0.51±0.01ab	0.52±0.01abcd	0.52
	Mean (S)	0.60	0.61		0.51A	0.54B	
	LSD ($P \leq 0.05$)	T=ns	S=ns	TXS=ns	T=ns	S=0.018	TXS=0.37
SSC: TA	Control	19.69±1.18	18.79±1.06	19.24A	24.98±0.62	21.59±0.52	23.29
	BC	19.79±0.23	20.08±0.50	19.93A	24.40±0.14	23.00±0.44	23.70
	NC	19.96±0.99	23.04±0.48	21.50B	24.22±0.46	22.63±0.33	23.43
	1-MCP	21.07±0.37	20.11±0.27	20.59AB	25.69±0.43	23.31±0.60	24.50
	Mean (S)	20.13	20.50		24.82B	22.64A	
	LSD ($P \leq 0.05$)	T=1.47	S=ns	TXS=ns	T=ns	S=0.84	TXS=ns

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), Mean ± SE. The Duncan's multiple range test at ($P \leq 0.05$) was used to test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage means. 90 and 120 days data were analysed separately.

with 1-MCP and stored in ozonised cold storage exhibited significantly highest SSC: TA values (28.82) (Table 5.5).

5.3.2.4 Individual sugars

The Cripps Pink and Granny Smith fruit fumigated with 1-MCP and cold-stored for 90 and 120 days recorded significantly higher mean levels of glucose and lowest mean sucrose levels when compared to control, NC and BC treatments (Table 5.7 and 5.8). The mean fructose levels in Cripps Pink apple were significantly lowest (313.7 g kg^{-1}) in the fruit treated with NC and cold-stored for 90 days when compared to control and other ethylene antagonist treatments but showed no significant effect in 120 days stored fruit. (Table 5.7). The Granny Smith fruit fumigated with 1-MCP exhibited significantly highest values of sorbitol in 90 days cold-stored fruit, whilst they were significantly lowest in 120 days cold-stored fruit (Table 5.8). The control and BC treated Cripps Pink fruits recorded higher mean levels of sorbitol when compared to NC and 1-MCP (Table 5.7). The Granny Smith fruit fumigated with NC exhibited significantly higher mean sorbitol levels when compared to other treatments (Table 5.8).

The Cripps Pink fruit stored for 90 days in ozonised cold storage exhibited higher mean values of glucose (18.17 g kg^{-1}), fructose (344.3 g kg^{-1}), sorbitol (27.15 g kg^{-1}) and lower sucrose (273.02 g kg^{-1}). Whilst, 120 days the mean levels of the sucrose (215.97 g kg^{-1}) was significantly higher in the fruit stored in ozonised cold storage (Table 5.7). The ozone application in the cold storage did not significantly affect the mean levels of glucose, fructose and sorbitol in the Cripps Pink fruit stored for 120 days. The ozonised cold storage did not show a significant effect on the levels of glucose and sucrose in Granny Smith fruit for both 90 and 120 days of storage. The levels of sorbitol were comparatively lower in the Granny Smith fruit stored in ozonised cold storage for both 90 (14.34 g kg^{-1}) and 120 (18.72 g kg^{-1}) days. The Granny Smith stored in ozonised cold storage for 90 days showed significantly higher fructose levels, but there was no significant effect of the presence of ozone in 120 days cold-stored fruit.

The interaction effect between ozone application in cold storage and the ethylene antagonist fumigation on the Cripps Pink apple fruit was significant in case of the

sucrose levels for both 90 and 120 days storage durations. Other than that, the interaction was also significant for the glucose and sorbitol levels in the Cripps Pink fruit stored for 120 days (Table 5.7). The Cripps Pink fruit treated with 1-MCP and stored in cold storage without ozone application showed significantly lowest values of sucrose for both 90 (264.4 g kg^{-1}) and 120 (177.77 g kg^{-1}) days storage when compared to all other conditions.

The interaction between ozone application in cold storage and the ethylene antagonist fumigation exhibited a significant effect on the sorbitol levels of Granny Smith fruit cold-stored for 90 and 120 days. The 1-MCP fumigated Granny Smith fruit stored in ozonised cold storage showed significantly lowest sorbitol levels compared to all other conditions for both 90 (13.81 g kg^{-1}) and 120 (15.30 g kg^{-1}) days storage (Table 5.8).

5.3.2.5 Individual organic acids

The fruit pulp samples investigated using HPLC system exhibited considerable amounts of malic acid and succinic acid, very low amounts of fumaric acid, tartaric acid and no citric acid. The fumigation treatment with 1-MCP retained significantly highest mean amounts of succinic acid when compared to other treatments in both 90 and 120 days cold-stored Cripps Pink and Granny Smith fruit followed by NC fumigation treatment (Table 5.9 and 5.10).

The Granny Smith and Cripps Pink fruit stored in ozonised cold storage for 90 and 120 days showed reduced levels of succinic acid (Table 5.9 and 5.10). The levels of malic acid were significantly higher in the Granny Smith fruit stored for 90 (4.35 g kg^{-1}) and 120 (4.96 g kg^{-1}) days in ozonised cold storage (Table 5.10).

The interaction effect between ethylene antagonist fumigation and the ozone application treatment was significant in the case of succinic acid levels of apple fruit cold-stored for 90 and 120 days. The Cripps Pink and Granny Smith apple fruit fumigated with 1-MCP and stored in non-ozonised cold storage for 90 and 120 days exhibited the highest levels of succinic acid (Table 5.9 and 5.10).

Table 5.7 Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0 ± 2 °C) with or without ozone gas on the levels of individual sugars (g kg^{-1}) in the pulp of Cripps Pink apple fruit

		Storage period (days)					
		90			120		
Sugars	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
Glucose	Control	0.00±0.0	19.49±5.68	9.74A	6.48±2.48ab	2.51±2.18a	4.49A
	BC	4.81±4.17	12.37±3.68	8.59A	11.46±1.19bc	19.08±0.27d	15.27B
	NC	0.0±0.0	12.54±4.00	6.27A	11.10±1.36bc	17.41±0.65d	14.26B
	1-MCP	9.86±5.03	28.26±3.76	19.06B	19.53±1.73d	12.13±0.71c	15.83B
	Mean (S)	3.66A	18.17B		12.14	12.78	
	LSD ($P \leq 0.05$)	T=9.12	S=6.45	TXS=ns	T=3.52	S=ns	TXS=4.98
Fructose	Control	329.68±6.88	361.89±5.07	345.79B	249.38±3.79	246.48±3.35	247.93
	BC	320.68±3.08	347.20±3.92	333.94B	244.15±0.45	256.42±3.02	250.28
	NC	308.83±3.80	318.62±3.27	313.73A	255.35±4.37	256.11±1.17	255.73
	1-MCP	322.05±3.22	349.34±10.62	335.70B	247.84±3.14	245.76±3.90	246.80
	Mean (S)	320.31A	344.26B		249.18	251.19	
	LSD ($P \leq 0.05$)	T=12.54	S=8.87	TXS=ns	T=ns	S=ns	TXS=ns
Sucrose	Control	309.82±6.03c	260.18±4.73a	285.00B	209.25±2.99cd	244.09±3.22e	226.67B
	BC	307.47±5.03bc	288.20±4.08b	297.83C	207.81±4.02bcd	195.14±2.24b	201.48A
	NC	303.78±3.07bc	292.43±2.37bc	298.10C	208.19±3.41bcd	205.01±5.00bc	206.60A
	1-MCP	264.35±4.24a	251.26±11.15a	257.80A	177.77±3.08a	219.63±3.66d	198.70A
	Mean (S)	296.35B	273.02A		200.75A	215.97B	
	LSD ($P \leq 0.05$)	T=12.78	S=9.04	TXS=18.07	T=8.72	S=6.17	TXS=12.33
Sorbitol	Control	31.02±1.70	29.66±1.72	30.34B	20.68±0.54bc	23.21±0.57d	21.94C
	BC	27.65±0.50	30.75±1.35	29.20B	22.30±0.14cd	22.99±0.70d	22.64C
	NC	23.42±0.37	23.25±0.58	23.34A	20.84±0.52bc	20.00±0.51b	20.42B
	1-MCP	25.19±0.72	24.93±2.92	25.06A	19.01±0.60b	16.71±0.89a	17.86A
	Mean (S)	26.82	27.15		20.70	20.73	
	LSD ($P \leq 0.05$)	T=3.62	S=ns	TXS=ns	T=1.42	S=ns	TXS=2.31

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), mean ± SE. Duncan's multiple range test at ($P \leq 0.05$) was used to test the mean separation for significant analysis of variance. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage means. 90 and 120 days data were analysed separately.

Table 5.8 The levels of individual sugars (g kg^{-1}) influenced by the ethylene antagonists in the pulp of Granny Smith apple fruit stored in cold storage (0 ± 2 °C) for 90 and 120 days

		Storage period (days)					
		90			120		
Sugars	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
Glucose	Control	98.79±8.66a	91.78±7.67a	95.29A	164.18±7.83	136.39±7.13	150.28A
	BC	116.18±9.94ab	108.50±6.65ab	112.34A	171.18±10.77	183.27±4.56	177.23B
	NC	147.02±19.67bc	146.04±14.42bc	146.53B	153.23±7.28	152.39±7.52	152.81A
	1-MCP	169.23±5.44c	237.89±5.13d	203.56C	219.33±5.00	217.54±6.69	218.44C
	Mean (S)	132.80	146.06		176.98	172.40	
	LSD ($P \leq 0.05$)	T=27.17	S=ns	TXS=38.42	T=18.04	S=ns	TXS=ns
Fructose	Control	381.02±4.98	392.48±2.19	386.75A	470.19±17.96	507.14±16.25	488.66B
	BC	371.24±5.23	390.15±7.18	380.70A	485.15±9.54	451.65±4.44	468.40AB
	NC	391.39±5.82	404.88±1.13	398.13B	451.37±13.22	466.57±4.03	466.37AB
	1-MCP	398.99±3.88	411.05±4.43	405.02B	466.18±7.94	446.98±5.73	449.18A
	Mean (S)	385.66A	399.64B		468.22	468.08	
	LSD ($P \leq 0.05$)	T=11.17	S=7.90	TXS=ns	T=27.11	S=ns	TXS=ns
Sucrose	Control	166.02±12.65	180.73±10.82	173.38B	168.98±5.71bc	202.78±12.01d	185.88B
	BC	171.23±6.88	172.53±8.94	171.88B	184.41±6.67cd	157.081±5.25b	170.74B
	NC	156.90±13.68	160.56±9.02	158.73B	187.90±6.35cd	188.09±6.19cd	187.99B
	1-MCP	136.56±3.17	120.04±0.70	128.30A	130.95±4.17a	130.01±4.91a	130.48A
	Mean (S)	157.68	158.47		168.06	169.49	
	LSD ($P \leq 0.05$)	T=22.51	S=ns	TXS=ns	T=17.03	S=ns	TXS=24.09
Sorbitol	Control	14.11±0.35a	13.63±0.09a	13.87A	21.74±1.09c	22.80±0.60c	22.27C
	BC	17.47±0.58c	14.22±0.48a	15.84B	21.97±0.76c	18.02±0.72b	20.00B
	NC	18.76±0.43c	15.69±0.26b	17.23C	22.79±0.84c	18.77±0.29b	20.78BC
	1-MCP	15.64±0.18b	13.81±0.36a	14.73A	19.14±0.50b	15.30±0.58a	17.22A
	Mean (S)	16.49B	14.34A		21.41B	18.72A	
	LSD ($P \leq 0.05$)	T=0.93	S=0.66	TXS=1.32	T=1.81	S=1.28	TXS=2.55

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), ± SE. The Duncan's multiple range test at ($P \leq 0.05$) was used to test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage means. 90 and 120 days data were analysed separately

Table 5.9 Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0 ± 2 °C) with or without ozone gas on the levels of individual organic acids (g kg^{-1}) in the pulp of Cripps Pink apple fruit

		Storage period (days)					
		90			120		
Organic acids	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
Malic acid	Control	5.64±0.26	5.03±0.24	5.33	6.49±0.17a	6.17±0.10a	6.33AB
	BC	5.28±0.18	5.08±0.07	5.18	6.09±0.36a	6.34±0.13a	6.21A
	NC	5.57±0.30	5.45±0.25	5.51	7.44±0.25b	5.87±0.11a	6.65B
	1-MCP	5.33±0.33	5.79±0.19	5.56	6.21±0.08a	5.89±0.04a	6.05A
	Mean (S)	5.45	5.34		6.56B	6.07A	
	LSD ($P \leq 0.05$)	T=ns	S=ns	TXS=ns	T=0.42	S=0.29	TXS=0.59
Succinic acid	Control	0.74±0.02bc	0.61±0.02a	0.67A	0.79±0.03c	0.73±0.01bc	0.76B
	BC	0.61±0.02a	0.66±0.0ab	0.63A	0.68±0.04ab	0.66±0.01a	0.67A
	NC	0.75±0.03bc	0.64±0.02a	0.69A	0.86±0.02c	0.76±0.01c	0.78B
	1-MCP	0.79±0.04c	0.76±0.02c	0.77B	0.95±0.01d	0.80±0.01c	0.88C
	Mean (S)	0.72B	0.67A		0.81B	0.74A	
	LSD ($P \leq 0.05$)	T=0.06	S=0.04	TXS=0.09	T=0.05	S=0.03	TXS=0.07

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range test at ($P \leq 0.05$) was used to test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage means. 90 and 120 days data were analysed separately

Table 5.10 The levels of individual organic acids (g kg^{-1}) influenced by the ethylene antagonists in the pulp of Granny Smith apple fruit stored in cold storage (0 ± 2 °C) for 90 and 120 days

		Storage period (days)					
		90 Days			120 Days		
Organic acids	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
Malic acid	Control	3.25±0.31a	4.15±0.19bc	3.70	4.52±0.11	4.95±0.21	4.74
	BC	3.51±0.33ab	4.65±0.04c	4.08	4.93±0.10	4.93±0.14	4.93
	NC	4.23±0.31bc	4.60±0.09c	4.41	4.64±0.18	4.95±0.31	4.80
	1-MCP	4.38±0.25c	4.00±0.25abc	4.19	4.60±0.18	5.00±0.15	4.80
	Mean (S)	3.84A	4.35B		4.67A	4.96B	
	LSD ($P \leq 0.05$)	T=ns	S=0.38	TXS=0.77	T=ns	S=0.24	TXS=ns
Succinic acid	Control	0.42±0.03ab	0.41±0.01a	0.4153A	0.57±0.02bc	0.48±0.01a	0.53A
	BC	0.50±0.03bc	0.42±0.01ab	0.4608AB	0.58±0.01c	0.50±0.01ab	0.54A
	NC	0.55±0.03c	0.44±0.01ab	0.4939B	0.68±0.04d	0.48±0.02a	0.58A
	1-MCP	0.73±0.03d	0.42±0.02ab	0.5736C	0.77±0.04e	0.52±0.01abc	0.64B
	Mean (S)	0.55B	0.42A		0.65B	0.49A	
	LSD ($P \leq 0.05$)	T=0.05678	S=0.04	TXS=0.08	T=0.055	S=0.04	TXS=0.08

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range test at ($P \leq 0.05$) was used to test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage means. 90 and 120 days data were analysed separately.

5.3.2.6 Total phenols

The Cripps Pink fruit fumigated with NC exhibited significantly higher mean levels of total phenols during 90 (33.12 g GAE kg^{-1}) and 120 (40.61 g GAE kg^{-1}) days cold storage when compared to control and other fumigation treatments (Table 5.11). Whilst fumigation treatments did not show any significant effect on total phenol levels in the Granny Smith fruit cold-stored for 90 and 120 days (Table 5.12).

The Cripps Pink and Granny Smith fruit stored in ozonised cold storage recorded higher mean total phenols levels at 90 days storage, but at 120 days storage in ozonised cold storage the levels of total phenols in the fruit were lower than non-ozonised one.

There was a significant interaction effect between ethylene antagonist treatments and ozone gas application on the levels of total phenols for 90 days cold storage, but it was not seen at 120 days storage. The fruits fumigated with NC (39.20 g GAE kg^{-1}) and 1-MCP (40.33 g GAE kg^{-1}) stored in ozonised cold storage showed significantly highest amounts following 90 days storage.

5.3.2.7 Ascorbic acid

The Cripps Pink and Granny Smith apple fruit fumigated with NC and MCP showed lower levels of ascorbic acid when compared to fruits fumigated with BC and control. The changes in ascorbic acid levels were significant only for the fruit cold-stored for 90 days in Cripps Pink apple, while in case of Granny Smith apple the changes were significant only in the fruit cold-stored for 120 days (Table 5.11 and 5.12).

Overall, the Cripps Pink fruit stored in ozonised cold storage exhibited higher mean ascorbic acid content in both 90 (11.96 g kg^{-1}) and 120 (13.13 g kg^{-1}) days cold-stored fruit (Table 5.11), whilst for the Granny Smith apple, the presence of ozone did not show any significant effect on levels of ascorbic acid.

The interaction effects of fumigation treatments and ozone application on levels of ascorbic acid were significant only for the fruit cold-stored for 90 days in Cripps Pink apple, while only 120 days stored Granny Smith apple fruit had significant interaction

effect. The changes due to interaction effect did not follow any specific trend in both the cultivars studied.

5.3.2.8 Total antioxidant capacity

The fruit fumigated with 1-MCP exhibited higher levels of total antioxidant capacity when compared to all other treatments in both Cripps Pink and Granny Smith apple fruit cold-stored for 90 and 120 days (Table 5.11 and 5.12).

The Cripps Pink apple fruit stored in ozonised cold storage had higher levels of total antioxidant capacity but contrarily the Granny Smith stored in ozonised cold storage exhibited lower mean values of total antioxidant capacity when compared to the fruit stored in cold storage without ozone (Table 5.11 and 5.12).

There was no significant interaction effect between ethylene antagonist fumigation and ozone application on levels of total antioxidant capacity of Cripps Pink apple fruit stored for 90 and 120 days and Granny Smith apple stored for 90 days in cold storage.

Table 5.11 Influence of BC, NC and 1-MCP fumigation followed by 90 and 120 days of cold storage (0 ± 2°C) with or without ozone gas on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of Cripps Pink apple fruit

		Storage period (days)					
		90			120		
Parameters	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
Total phenols (g GAE kg^{-1})	Control	25.08±0.89a	22.08±0.89a	23.58A	28.53±1.18	23.49±2.51	26.01A
	BC	26.39±2.99a	24.33±1.60a	25.36A	29.29±2.74	28.35±2.83	28.82A
	NC	27.04±2.22a	39.20±2.24b	33.12B	39.11±3.21	42.10±0.61	40.61B
	1-MCP	27.23±3.42a	40.33±2.67b	33.78B	29.57±2.18	29.66±0.84	29.61A
	Mean (S)	26.43A	31.48B		31.62	30.90	
	LSD ($P \leq 0.05$)	T=5.48	S=3.88	TXS=7.76	T=4.99	S=ns	TXS=ns
Ascorbic acid (g kg^{-1})	Control	11.21±0.19a	12.15±0.39b	11.68BC	12.33±0.46	12.08±0.16	12.20
	BC	11.26±0.20a	13.20±0.26c	12.23C	13.22±0.51	13.20±0.28	13.21
	NC	10.70±0.06a	11.19±0.14a	10.95A	13.16±0.50	13.57±0.23	13.37
	1-MCP	11.35±0.12ab	11.28±0.35a	11.31AB	12.73±0.20	13.65±0.30	13.19
	Mean (S)	11.13A	11.96B		12.86	13.13	
	LSD ($P \leq 0.05$)	T=0.57	S=0.40	TXS=0.81	T=ns	S=ns	TXS=ns
Total antioxidant capacity (µM kg^{-1} Trolox)	Control	8.82±0.21	11.02±0.07	9.92A	10.69±0.07	11.06±0.11	10.87A
	BC	9.12±0.12	11.98±0.33	10.55B	10.88±0.09	11.24±0.13	11.06AB
	NC	8.53±0.29	10.74±0.33	9.63A	11.50±0.33	11.79±0.32	11.65B
	1-MCP	8.73±0.13	11.50±0.14	10.11AB	11.40±0.45	11.85±0.14	11.62B
	Mean (S)	8.80A	11.31B		11.12	11.48	
	LSD ($P \leq 0.05$)	T= 0.53	S=0.37	TXS= ns	T=0.60	S=ns	TXS=ns

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range test at ($P \leq 0.05$) was used to test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage means. 90 and 120 days data were analysed separately.

Table 5.12 The levels of total phenols, ascorbic acid and total antioxidant capacity influenced by the ethylene antagonists in the pulp of Granny Smith apple fruit stored in cold storage (0 ± 2°C) for 90 and 120 days

		Storage period (days)					
		90 Days			120 Days		
	Treatment	Non-Ozone	Ozone	Mean (T)	Non-Ozone	Ozone	Mean (T)
Total phenols (g GAE kg^{-1})	Control	58.01±3.35b	57.82±4.93b	57.92	84.49±5.62	70.27±1.96	77.38
	BC	56.79±2.68b	56.14±2.49b	56.47	76.44±10.12	59.69±1.40	68.07
	NC	56.14±4.63b	58.29±5.10b	57.22	80.00±5.56	67.37±4.50	73.68
	1-MCP	42.01±1.16a	66.62±2.94b	54.31	86.92±3.71	80.28±3.16	83.60
	Mean (S)	53.24A	59.72B		81.96B	69.40A	
	LSD ($P \leq 0.05$)	T=ns	S=6.00	TXS=12.00	T=ns	S=8.34	TXS=ns
Ascorbic acid (g kg^{-1})	Control	15.07±0.32	13.88±0.26	14.48	14.60±0.20bc	15.36±0.15c	14.98C
	BC	14.34±0.46	14.09±0.03	14.22	15.33±0.38c	13.92±0.34ab	14.62BC
	NC	14.81±0.63	13.62±0.24	14.21	13.41±0.12a	13.62±0.21a	13.52A
	1-MCP	13.88±0.28	13.90±0.55	13.89	13.60±0.41a	14.93±0.42c	14.27B
	Mean (S)	14.52	13.88		14.24	14.46	
	LSD ($P \leq 0.05$)	T=ns	S=ns	TXS=ns	T=0.62	S=ns	TXS=0.87
Total antioxidant capacity (µM kg^{-1} Trolox)	Control	12.68±0.45	10.67±0.33	11.68A	12.62±0.48ab	13.06±0.38ab	12.84
	BC	12.24±0.22	10.60±0.54	11.42A	13.88±0.10b	11.84±0.12a	12.86
	NC	12.48±0.13	10.71±0.39	11.59A	12.66±0.51ab	12.66±0.26ab	12.66
	1-MCP	13.83±0.45	11.96±0.24	12.90B	13.30±0.26b	13.56±0.47b	13.43
	Mean (S)	12.81B	10.99A		13.12	12.78	
	LSD ($P \leq 0.05$)	T= 0.92	S=0.65	TXS= ns	T= ns	S=ns	TXS= 1.21

ns = non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range test at ($P \leq 0.05$) was used to test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage means. 90 and 120 days data were analysed separately.

5.4 Discussion

The effects of ozone in combination with new ethylene antagonists (BC and NC) and 1-MCP on the postharvest physiology and fruit quality have been investigated for the first time in the cold-stored Cripps Pink and Granny Smith apples. Several ripening-related physical, biochemical and physiological changes in fruits are regulated by the gaseous hormone ethylene (Imaseki, 1991; Anwar et al, 2018). The fumigation treatment with BC, NC and 1-MCP were effective in significantly reducing the rates of ethylene biosynthesis, respiration and in delaying ripening-related changes. This indicates that even the new ethylene antagonists effectively bind to the ethylene receptors to inhibit the action and autocatalytic production of ethylene in the fruit (Sisler, 2006). Ozone gas is well known for its wide-spectrum antimicrobial nature but its effects on fruit physiology and quality depend upon several factors such as fruit variety, ozone concentrations, duration of treatment and storage temperatures (Tokala et al., 2018).

The rates of the respiratory and ethylene climacteric peaks were reduced in the Cripps Pink and Granny Smith apple fruits fumigated with BC, NC and 1-MCP when compared to the untreated fruit. Similarly, the onset of these peaks was also delayed in the fruit fumigated with the ethylene antagonists. The inhibitory action of these compounds is due to the ability of the 1-alkyl cyclopropenes to attach with ethylene receptors and counteract both internal and external ethylene in the fruits (Sisler et al., 2003; Apelbaum et al., 2008). A similar mode of action for ethylene antagonistic activity of BC and NC was proposed by Musa (2016). The rate of ethylene climacteric peak was higher in the cold-stored fruit with ozone when compared to the fruit stored in cold storage without ozone. Ozone gas is a highly oxidising agent and high concentrations can induce oxidative stress in the plant tissues (Pell et al., 1997). This rise could be attributed to a stress-induced increase in ethylene production at high ozone concentrations (Liew and Prange, 1994).

The Cripps Pink and Granny Smith apple fruit fumigated with BC, NC and 1-MCP exhibited lower PLW when compared to control fruit. Generally, the fruit loses water during storage and in turn show a reduction in their weight as a result of physiological activities such as respiration and transpiration (Becker and Fricke, 1996). The lowering

of PLW in ethylene antagonist fumigated fruits could be related to the reduction in rates of respiration and ethylene production (Martínez-Romero et al., 2007). The Cripps Pink and Granny Smith apple stored in ozonised cold storage showed a reduction in PLW during storage. Similar observations were also recorded in other apple cultivars (Bazarova, 1982) as well as in other fruits such as strawberries (Zhang et al., 2011) and papaya (Ali et al., 2014).

Retention of fruit firmness in apples during storage is one of the important characteristics for consumer acceptability (DeEll et al., 1999). The average ideal fruit firmness of Cripps Pink apple is 58 N, while it is 44 N for Granny Smith apple. The fruit firmness was maintained high in the Cripps Pink and Granny Smith apple fruit fumigated with BC, NC and 1-MCP when compared to the control fruit. Loss of fruit firmness during storage occurs due to loss of cell turgor and cell wall metabolism. Cell wall hydrolysing enzymes in the fruit are activated chiefly by the involvement of ethylene hormone (Giovannoni, 2008). The maintenance of fruit firmness can be associated with lowered rates of ethylene production and reduction in PLW by the ethylene antagonistic treatments (Giovannoni, 2008). Application of ozone gas during cold storage of Cripps Pink and Granny Smith apple did not show a consistent effect on fruit firmness. Even Skog and Chu (2001) reported that ozone application did not show any effect on the firmness of apple fruit.

The SSC was reduced in the Cripps Pink apples fumigated with BC, NC and 1-MCP, whilst they were higher in case of Granny Smith apples. The contrasting results of ethylene antagonist application with different apple varieties were also reported by Blankenship and Dole (2003) and the opined that the effects could be varietal specific. This also indicates that the accumulation of SSC in the apple fruit is not necessarily associated with the perception of the ethylene (Fan et al., 1999). There was no significant effect or a distinctive trend on levels of TA with ethylene antagonist (BC, NC and 1-MCP) treatments or by storage environment in both Cripps Pink and Granny Smith apple fruit. Similar observations were reported by Mir et al. (2001) in Redchief Delicious apple fruit and by Watkins et al. (2000) in McIntosh, Empire, Delicious and Law Rome apple fruit when treated with 1-MCP under different storage environments.

The levels of glucose were higher in the Cripps Pink and Granny Smith apple fruit fumigated with 1-MCP but at the same time levels of other sugars were lower when compared to other treatments. These variations are due to the interconversion of sugars during the ripening process (Ackermann et al., 1992). Random fluctuations in levels of different sugars were observed with different ethylene antagonist treatments. These changes could be attributed to different conversions occurring among glucose, fructose, sucrose and sorbitol during the ripening process and also as a response to the storage environment (Ackermann et al., 1992). The levels of glucose, fructose and sucrose were higher in the Cripps Pink and Granny Smith apple fruit stored in ozonised cold storage. Similar observations were previously reported by Glowacz et al. (2015b) and Aguayo et al. (2006).

The fruit ripening process is considered an oxidative process involving several alterations including changes in the reactive oxidative species (ROS) (Masia, 1998). The ethylene gas is responsible for reactions involving ROS generation during the ripening process (Steinite et al., 2004). The major constituents of bioactive compounds in the apple fruit are total phenols, ascorbic acids and flavonols (Łata and Tomala, 2007). During fruit ripening process the phenolics and other antioxidant compound are depleted in order to degrade the ROS produced (Valero et al., 2016). The Cripps Pink and Granny Smith apple fruit treated with ethylene antagonists exhibited higher levels of total phenols and total antioxidant capacity when compared to control. These changes could be ascribed due to a reduction in rates of ethylene production (Masia, 1998). The ascorbic acid levels in the Cripps Pink and Granny Smith apple treated with ethylene antagonist were lower in comparison with the control fruit. Vilaplana et al. (2006) also recorded a similar reduction in cold-stored apple fruit treated with 1-MCP.

5.5 Conclusions

The new ethylene antagonists (BC and NC) and 1-MCP were effective in antagonising the ethylene effects and maintaining optimum quality in Granny Smith and Cripps Pink apple fruit. The Cripps Pink fruit fumigated with BC and NC and the Granny Smith fruit fumigated with 1-MCP exhibited the lowest respiration and ethylene climacteric peak rates when compared to other treatments. The ozone application in the cold storage helped

in maintenance of postharvest fruit quality but attributed to an increase in the rate of ethylene production. The Granny Smith and Cripps Pink apple fruit stored in cold storage with ozone showed comparatively lower PLW and higher levels of individual sugars. The interaction between the ethylene antagonists and ozone application during cold storage remained non-significant for most of the fruit physiological and quality parameters examined. The effects of ethylene antagonists and ozone exhibited similar trends for both 90 and 120 days of storage in almost all the parameters studied, with the exception in levels of individual sugars. The magnitude of the effects of the ethylene antagonist treatment varied in the two apple varieties studied. Effects of different concentrations of ozone on the fruit quality of apple varieties and standardising the optimum ozone concentrations warrants future investigation to efficiently exploit advantageous of ozone. Both NC and BC possess the capability to be used as an alternative to 1-MCP as ethylene antagonists.

CHAPTER 6

Effects of novel ethylene antagonists and AiroFresh® in regulating ethylene production and fruit quality of Cripps Pink apples stored in controlled atmosphere storage

Abstract

Regulating the process of ripening is considered as one of the important ways to extend the storage life and to reduce the postharvest losses while maintaining the optimum fruit quality. The plant hormone ethylene promotes different ripening-associated irreversible physical, physiological and biochemical changes in the fruit. In the present investigation, the effects of fumigation with two potential ethylene antagonists namely 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) as well as 1-methylcyclopropene (1-MCP) on the ethylene production, respiration and the postharvest quality of Cripps Pink apple fruit stored in controlled atmosphere (CA) storage with and without AiroFresh® technology were studied. The Cripps Pink apple fruit were fumigated with ethylene antagonists for 18 h in the hermetically sealable 60 L plastic drums. The untreated fruit were considered as the control. The treated fruit were stored for 90 and 120 days in CA storage installed with AiroFresh® (ACA) and conventional CA storage. All the ethylene antagonist (BC, NC and 1-MCP) treatments significantly lowered the rates of respiration and ethylene production when compared to the control fruit during 90 and 120 days of storage. The installation of AiroFresh® technology in the CA storage showed a synergistic effect in retarding the rates of the ethylene and respiration climacteric peaks as well as in maintaining the postharvest fruit quality when compared to conventional CA storage following 90 days, but the efficiency reduced following 120 days of storage. The fumigation with NC exhibited comparatively better results than BC and 1-MCP, in lowering ethylene and respiration rates, reducing PLW and in maintaining higher levels of fruit firmness, total phenol and total antioxidant capacity during controlled atmosphere storage with or without AiroFresh®.

6.1 Introduction

Ripening is a natural process in the fruits and is considered as one of the important and inherent causes of the postharvest losses. The ripening process is the final stage of fruit development involving several catabolic and anabolic reactions. It is associated with different interdependent physiological and biochemical changes in the fruit which ultimately cause senescence and deterioration. The sequence of irreversible reactions during ripening leads to hydrolysis of cell wall materials, conversion of starch into sugars, degradation of chlorophyll pigments, development of aroma volatiles and depletion of bioactive compounds (Tucker, 2012).

The phytohormone ethylene is produced by almost all the plant parts and is responsible for promoting the fruit ripening processes (Burg and Burg, 1962; Anwar et al., 2018). Apples are categorised as climacteric fruit based upon the respiration and ethylene production trends during the ripening process. They exhibit a distinctive sharp rise in the respiration and ethylene production during the ripening process and this rise is termed as 'climacteric peak' (Giovannoni, 2008). Apple fruit are very sensitive to the external ethylene exposure and in response, they show rapid changes in fruit physiology and also produce large amounts of internal ethylene. The ripening-associated processes directly or indirectly affect the postharvest fruit quality and storage life of the apple fruit (Abeles et al., 1992). Slowing down the process of ripening by appropriate postharvest handling, regulating ethylene and maintaining optimum storage conditions can considerably extend storage life, reduce the postharvest losses and maintain optimum fruit quality (Gross et al., 2016). Several methods are being adapted to regulate ethylene to in turn extend storage life and maintain fruit quality. The fumigation treatment with 1-alkylcyclopropenes is one of the best ways to effectively antagonise ethylene action in the fruit during storage (Watkins, 2006; Apelbaum et al., 2008). 1-MCP is relatively stable in the gaseous state compared to all other identified CP. The commercial formulation of 1-MCP is widely being used in the horticulture industry to extend the storage life of different fruit and vegetables (Valero et al., 2016).

The controlled atmospheric (CA) storage is being used commercially in different parts of the world to significantly extend the storage life of the apple fruit. In addition to the lowest

possible temperatures and high relative humidity (RH), the CA storage environment contains high levels of CO_2 and low O_2 levels when compared to the normal atmosphere. The rate of enzyme activities associated with fruit ripening is significantly reduced with the reduction in storage temperatures. Thus, the storage life is extended by lowering ethylene and respiration rates, delaying fruit softening, decreasing water loss and maintaining nutritional and organoleptic properties of the fruits (Paull, 1999; Gross et al., 2016). Similarly, reducing O_2 levels and increasing CO_2 levels in the CA storage also significantly reduce ethylene and respiration rates in the fruit (Keller et al., 2013). The rates of ethylene production tend to increase in the apple fruit after removing from the CA storage. Treatment with ethylene antagonists along with CA storage help in retarding this sudden rise in ethylene production rates (Watkins, 2006; Bai et al., 2009).

AiroFresh® is a new technology developed by Creative Research Technology (CRT), South Australia, Australia. The manufacturer claims that it utilises advanced oxidation processes (AOP) and photocatalytic oxidation (PCO) principle to completely oxidise and degrade volatile organic compounds including ethylene in the closed storage environment. The technology claims to induce suitable conditions in the storage environment to enhance the efficiency of ethylene antagonists (AiroFresh®, 2019).

Although the 1-MCP is popular in the horticulture industry for its positive effects, yet it still has some limitations. The 1-MCP is unstable even at the temperatures as low as 0°C and vaporises immediately (Sisler et al., 2006). Commercially it is available only as a service, which is expensive to afford and not easily available to all the fruit growers. There is a need to explore possibilities to identify and develop compounds which could address these limitations. There are no studies conducted to investigate effects of installing AiroFresh® technology in the CA storage in combination with ethylene antagonist treatments on the rates of respiration and ethylene production as well as the quality of Cripps Pink apple fruit. The present experiment was designed to study the effects of two newly developed ethylene antagonists (BC and NC) and 1-MCP as well as AiroFresh® technology in retarding ethylene production and maintaining the fruit quality of Cripps Pink apples stored in CA storage for 90 and 120 days. It was hypothesised that the combination of ethylene antagonists and AiroFresh® during CA storage would

synergistically contribute to reducing ethylene and respiration rates as well as in maintaining the apple fruit quality.

6.2 Material and methods

6.2.1 Fruit and experimental conditions

The Cripps Pink apple fruit used for the experiment were sourced from Manjimup (34°13' S latitude, 116°08' E longitude) at the commercial harvest (fruit firmness 65.70 ± 6.42 N; SSC 10.93 ± 0.05 % and TA 0.61 ± 0.03 %) during 17th May 2017. The Cripps Pink trees were 11 years old and grafted on M26 rootstock and trained in modified central system. The trees were planted in North-South orientation with a spacing of 4.5 m (within the rows) and 1 m (between the rows). The fruit were given a postharvest fungicidal dip in an aqueous solution of 'Caltop' (a.i. 165 g L^{-1} calcium), 'Scholar' (a.i. 230 g L^{-1} fludioxonil), 'DPA' (diphenylamine) in order to control any postharvest diseases and disorders during storage. The fruit were air-dried till there are no water droplets left on the fruit surface, and then packed in corrugated cardboard boxes with softboard trays. The packed boxes were then transported to the Curtin Horticulture Research Laboratory, Perth using the air-conditioned vehicle.

Uniform sized fruit without any bruises, mechanical injuries or symptoms of disease or disorder were chosen for the experiment. The Cripps Pink apple fruit were fumigated with the ethylene antagonists (BC, NC and 1-MCP) as detailed in Chapter 3, Section 3.3.1. The fumigation treatment was done for 18 h using 60 L hermetically sealable drums while keeping sixty apple fruit per drum. The control fruit were not treated with any compound. The experiment was laid in completely randomised design and each treatment was replicated four times with fifteen fruit per treatment. Following the treatment, the fruit were packed in corrugated cardboard boxes with respect to the treatments. The boxes were divided into two lots, each lot meant to be stored in controlled atmosphere storage with AiroFresh® (ACA) and controlled atmosphere storage without AiroFresh® (CA) at grower's property in Manjimup, WA. Each lot was again sub-divided into two sublots, with each to be stored for 90 and 120 days. The gas concentrations in CA were maintained at 2.40 ± 0.45 % of CO_2 and 3.25 ± 0.50 % of O_2 and temperature at 0 ± 1 °C. The CA

storage environments and the length of storage period were decided according to the commercial storage practices of the apple growers. The apple fruit were removed from the storage on the completion of designated storage periods and brought back to Curtin Horticulture Research Laboratory immediately for analysing different physiological and quality parameters of the fruit.

6.2.2 Determination of fruit physiological parameters

Following the assigned CA storage period, two fruit per experimental unit were selected randomly to determine rates of ethylene production and respiration.

6.2.2.1 Rate of ethylene production

The ethylene production rate was estimated from the 1 mL volume of headspace gas drawn from the 1 L hermetically sealed glass jar with selected apple fruit. The sample headspace gas collected was injected into the GC (6890N Network GC system, Agilent Technology, CA, USA) to quantify the amount of ethylene gas in the sample. The rate of ethylene gas production was estimated every day until a distinct climacteric peak was recorded. The detailed procedure and respective calculations to estimate rates of ethylene production are explained in Chapter 3, Section 3.5.1. The calculated rate of ethylene production is expressed as µmol C_2H_4 kg^{-1} h^{-1}.

6.2.2.2 Rate of respiration

The two selected fruit were incubated in the 1 L hermetically sealed glass jars for 1 h at room temperature (20±2°C). A 2 mL gas sample volume drawn from the headspace of individual glass jars were injected into Infrared gas analyser (Servomex Gas Analyser, 1450 Food Package Analyser, Servomex Limited, UK) to estimate the amount of CO_2 released by the incubated fruit. The procedure and calculations to estimate rates of respiration are explained in detail in Chapter 3, Section 3.5.2. The rate of respiration calculated is expressed as mmol CO_2 kg^{-1} h^{-1}.

6.2.3 Determination of fruit quality parameters

6.2.3.1 Physiological loss of weight (PLW)

The weight of fifteen fruit in each replication was recorded before transferring them to CA and ACA storage and noted as initial weight. The final weight was recorded on completion of respective storage periods. The PLW was then calculated using the formula detailed in Chapter 3, Section 3.6.1. The PLW calculated is expressed as %.

6.2.3.2 Fruit firmness

The fruit firmness of ten randomly selected fruit per replication was estimated using Texture Analyser (TA Plus, Ametek Lloyd Instruments Limited, UK). The calculations and procedure to determine fruit firmness is explained in detail in Chapter 3, Section 3.6.2. The fruit firmness calculated is expressed in newtons (N).

6.2.3.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA

The SSC, TA and SSC: TA values was estimated from the pooled fruit juice sample of cut slices of thirteen fruit per replication. SSC was determined using an infrared digital refractometer (Atago- Palette PR 101, Atago Co., Tokyo, Japan). The titration method was used to estimate the TA of the fruit juice sample. The detailed calculations of and procedures to estimate SSC, TA and SSC: TA were mentioned in Chapter 3, Section 3.6.3.

6.2.3.4 Individual sugars and organic acids

The individual sugars and organic acids levels were estimated using a reverse-phase HPLC system (Waters 1525, Milford Corporation, USA). The Dual λ UV absorbance detector (Water 2487, Milford Corporation, USA) at 214nm was used to determine individual organic acids, while individual sugars were determined by Refractive Index (RI) detector (Water 2414, Milford Corporation, USA). The description of instruments, procedures and calculations were explained in detail in Chapter 3, Section 3.6.5. The levels of sugars and organic acids are expressed as g kg^{-1}.

6.2.3.5 Total phenols

The Folin-Ciocalteu reagent method was used to determine the levels of total phenols in the fruit pulp samples. The detailed procedure of sampling and estimation were explained in Chapter 3, Section 3.6.6. The standard curve drawn from the pure gallic acid was used

to calculate the total phenol levels in samples and expressed as g GAE kg^{-1} fresh weight basis.

6.2.3.6 Ascorbic acid

The ascorbic levels in the fruit pulp samples were estimated using UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK) following the procedure detailed in Chapter 3, Section 3.6.7. The amounts of ascorbic acid in the fruit pulp sample was calculated using the standard curve using pure L-ascorbic acid and expressed as g kg^{-1} fresh weight basis.

6.2.3.7 Total antioxidant capacity

The DPPH method detailed in Chapter 3, Section 3.6.8 was used to estimate levels of total antioxidant capacity in the fruit pulp samples. The standard curve drawn using pure Trolox was used to calculate levels of total antioxidant capacity and is expressed as µM kg^{-1} Trolox.

6.2.4 Statistical analysis

The data recorded was statistically analysed using *GenStat* software version 14.0 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) through one-way or two-way analysis of variance (ANOVA). The tabulated results were mentioned as means ± standard errors (SE) of the means. The least significance difference (LSD) between treatments was determined using F-test with 5 % error probability and were compared using Duncan multiple comparison tests.

6.3 Results

6.3.1 Fruit physiological parameters

6.3.1.1 Ethylene (C_2H_4) production

The mean ethylene climacteric peak onset was significantly delayed by ethylene antagonist (BC, NC and 1-MCP) treatments when compared to control in Cripps Pink fruit following 90 and 120 days of storage (Table 6.1 and Figure 6.1). The fruit treated

with NC and 1-MCP exhibited significantly longer delayed mean C_2H_4 climacteric peak onset (13.88 days and 14.38 days, respectively) when compared to control and fruit treated with BC following 90 days of CA storage. In the case of 120 days stored fruit all the ethylene antagonist treatments significantly delayed the C_2H_4 climacteric peak onset when compared to control fruit (Table 6.1). The mean C_2H_4 climacteric peak onset was not significantly affected by storing Cripps Pink fruit in the CA or ACA storage. The interaction effect between the ethylene antagonist fumigation treatments and types of CA storage on the onset of C_2H_4 climacteric peak was also not significant.

The fumigation treatment with BC, NC and 1-MCP significantly reduced the C_2H_4 climacteric peak rate when compared to the control fruit following both 90 and 120 days of storage (Table 6.1 and Figure 6.1). The C_2H_4 climacteric peak rate was lowest in fruit fumigated with 1-MCP (0.15 µmol $kg^{-1}h^{-1}$) followed by fumigation with BC (0.34 µmol $kg^{-1}h^{-1}$) and NC (0.36 µmol $kg^{-1}h^{-1}$) in 90 days stored fruit. Whilst in case of the fruit stored for 120 days the 1-MCP (0.37 µmol $kg^{-1}h^{-1}$) and NC (0.39 µmol $kg^{-1}h^{-1}$) exhibited significantly lowest C_2H_4 climacteric peak rate followed by fruit fumigated with BC (0.61 µmol $kg^{-1}h^{-1}$) (Table 6.1). There was no significant effect of CA storage type on the C_2H_4 climacteric peak rates following both 90 and 120 days of storage. The interaction effect between ethylene antagonist treatments and CA storage type on the C_2H_4 climacteric peak rate was significant following 120 days of storage. The control fruit stored in both CA (1.29 µmol $kg^{-1}h^{-1}$) and ACA (1.13 µmol $kg^{-1}h^{-1}$) storage for 120 days exhibited significantly highest rates of C_2H_4 climacteric peak (Table 6.1).

6.3.1.2 Respiration (CO_2 production)

The respiratory climacteric peak onset was significantly delayed in the Cripps Pink fruit treated with BC, NC and 1-MCP when compared to control following both 90 and 120 days of storage. In case of the 90 days of storage, the mean respiratory climacteric peak onset was delayed longer in the fruit fumigated with NC (10.75 days) followed by BC (8.38 days) and 1-MCP (7.25 days). The fruit fumigated with BC, NC and 1-MCP (8.62, 10.88 and 11.38 days respectively) showed a significantly delayed respiratory climacteric peak onset when compared to control (3.12 days) following 120 days of storage (Table 6.1). The mean respiratory climacteric peak onset was significantly delayed in case of

fruit stored in ACA (9.25 days) when compared to CA (6.00 days) storage following 90 days, but there was no significant effect on the fruit following 120 days of storage (Table 6.1). The interaction effect between ethylene antagonist treatments and storage types was significant on the respiratory climacteric peak onset following both 90 and 120 days of storage. The control fruit stored in CA (2.00 days) showed earliest respiratory climacteric peak following 90 days of storage, while in case of 120 days storage the control fruit stored in ACA (2.75 days) showed the earliest peak when compared to all other treatments (Table 6.1).

The fruit treated with ethylene antagonists (BC, NC and 1-MCP) exhibited significantly lower respiratory climacteric peak rates when compared to control following both 90 days and 120 days of storage. The fruit fumigated with NC and 1-MCP showed significantly lowest respiratory climacteric peak rates following both 90 days (0.72 and 0.71 mmol $kg^{-1}h^{-1}$, respectively) and 120 days (0.63 and 0.65 mmol $kg^{-1}h^{-1}$, respectively) (Table 6.1). The values of respiratory climacteric peaks were significantly lowest in the fruit stored in ACA following 90 days (0.72 mmol $kg^{-1}h^{-1}$) but were higher following 120 days (0.82 mmol $kg^{-1}h^{-1}$) when compared to fruit stored in CA (Table 6.1). The interaction effect between ethylene antagonist treatment and storage type on rates of the respiratory climacteric peak was significant in case of the fruit following 90 days of storage. The control fruit stored in CA exhibited the highest rates of the respiratory climacteric peak (1.08 mmol $kg^{-1}h^{-1}$) when compared to all other treatments in 90 days stored fruit (Table 6.1). In case fruit stored for 120 days, the interaction effect was not significant on respiratory climacteric peak values.

Table 6.1. Effect of the ethylene antagonists and AiroFresh® on the climacteric peak onset (days) and peak rates of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) of the Cripps Pink apple fruit stored in CA storage for 90 and 120 days.

		Storage period (days)					
		90			120		
Parameters	Treatment	ACA	CA	Mean (T)	ACA	CA	Mean (T)
C_2H_4 climacteric peak onset	Control	9.00±0.41	7.25±1.03	8.12A	12.75±1.11	13.25±1.03	13.00A
	BC	12.00±1.22	12.50±0.96	12.25B	15.75±0.25	16.25±0.25	16.00B
	NC	14.50±0.29	13.25±0.75	13.88C	16.25±0.25	16.50±0.29	16.38B
	1-MCP	14.50±0.29	14.25±0.25	14.38C	16.00±0.00	17.00±0.00	16.50B
	Mean (S)	12.50	11.81		15.19	15.75	
	LSD ($P \leq 0.05$)	T=1.55	S=ns	TXS=ns	T=1.17	S=ns	TXS=ns
C_2H_4 climacteric peak rate	Control	1.14±0.10	1.30±0.08	1.22C	1.13±0.10c	1.29±0.09c	1.21C
	BC	0.36±0.07	0.32±0.13	0.34B	0.78±0.10b	0.43±0.06a	0.61B
	NC	0.24±0.05	0.48±0.11	0.36B	0.38±0.10a	0.39±0.11a	0.39A
	1-MCP	0.17±0.04	0.13±0.01	0.15A	0.47±0.09a	0.27±0.05a	0.37A
	Mean (S)	0.48	0.56		0.69	0.60	
	LSD ($P \leq 0.05$)	T=0.18	S=ns	TXS=ns	T=0.18	S=ns	TXS=0.26
Respiration climacteric peak onset	Control	6.25±1.60b	2.00±0.00a	4.12A	2.75±0.48a	3.50±0.96a	3.12A
	BC	11.75±0.75c	5.00±0.00b	8.38B	12.25±0.25c	5.00±1.22ab	8.62B
	NC	9.75±0.75c	11.75±0.75c	10.75C	11.25±1.89c	11.50±2.25c	10.88B
	1-MCP	9.25±1.75c	5.25±0.25b	7.25B	9.50±2.10bc	12.25±2.25c	11.38B
	Mean (S)	9.25B	6.00A		8.06	8.94	
	LSD ($P \leq 0.05$)	T=2.08	S=1.47	TXS=2.95	T=3.41	S=ns	TXS=4.82
Respiration climacteric peak rate	Control	0.84±0.04cd	1.08±0.03e	0.96C	1.12±0.06	0.95±0.05	1.03C
	BC	0.67±0.05a	0.93±0.04d	0.80B	0.80±0.04	0.79±0.07	0.79B
	NC	0.66±0.06a	0.79±0.03bc	0.72AB	0.66±0.02	0.59±0.05	0.63A
	1-MCP	0.71±0.04ab	0.71±0.02ab	0.71A	0.70±0.04	0.60±0.02	0.65A
	Mean (S)	0.72A	0.88B		0.82B	0.73A	
	LSD ($P \leq 0.05$)	T=0.08	S=0.06	TXS=0.11	T=0.09	S=0.07	TXS=ns

ns= non-significant, T = treatments, S = storage conditions, n = 4 replicates (2 fruit per replication), mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. 90- and 120-days data were analysed separately.

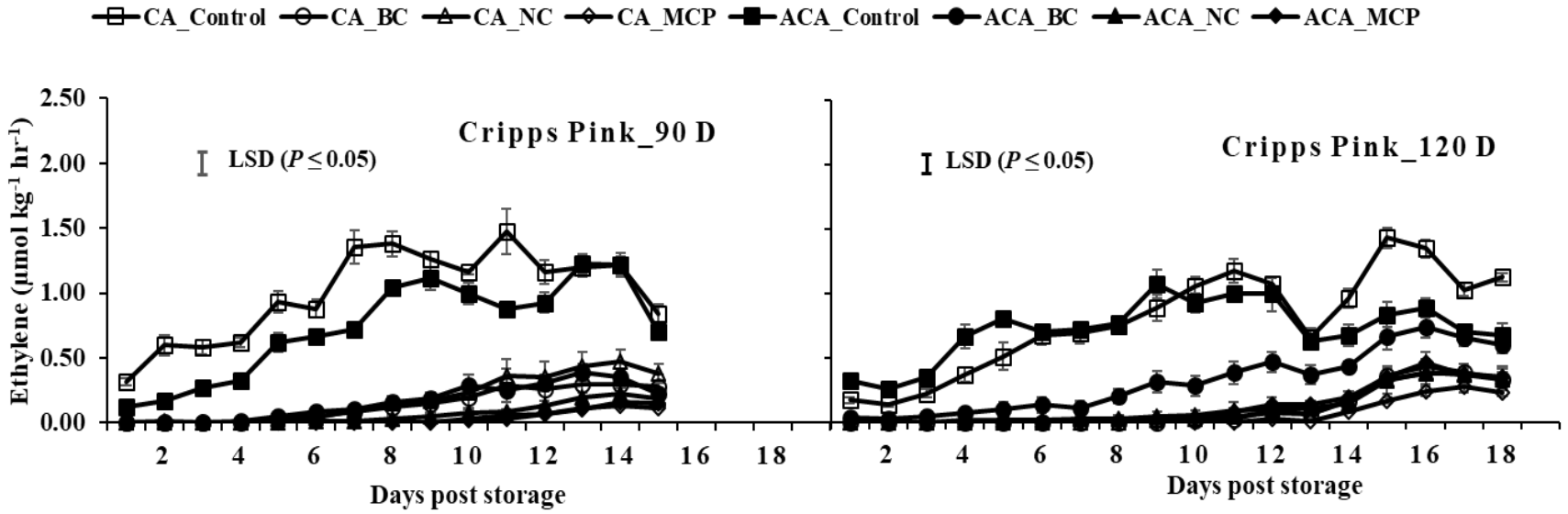


Figure 6.1 Changes in the rate of ethylene production due to ethylene antagonist fumigation treatment (T) days post storage (D) in Cripps Pink apple fruit stored for 90 and 120 days in CA and ACA storage. Vertical bars represent SE of mean values and are not visible when values are smaller than the symbol. n=4 replicates (2 fruit per replication). LSD ($P \leq 0.05$) T=0.05, D=0.06, TXD=0.17 for 90 days and T=0.04, D=0.05, TXD=0.16 for 120 days.

6.3.2 Fruit quality parameters

6.3.2.1 Physiological loss of weight (PLW)

The mean PLW in the fruit fumigated with ethylene antagonists (BC, NC and 1-MCP) was lower when compared to control fruit following both 90 and 120 days of storage. The fruit fumigated with BC and NC exhibited significantly lowest mean PLW (1.95 % and 2.12 %, respectively) following 120 days of storage when compared to fruit fumigated with 1-MCP and control fruit (Table 6.2). The mean PLW % values were significantly lowest (1.27 %) in the fruit stored in ACA following 90 days but the value is highest (2.48 %) following 120 days of storage when compared to fruit stored in CA (Table 6.2). No significant interaction effect between ethylene antagonist treatment and the storage type was observed during both 90 and 120 days of storage.

6.3.2.2 Fruit firmness

The fruit firmness in the Cripps Pink fruit fumigated with BC, NC and 1-MCP was maintained higher when compared to control following both 90 and 120 days of storage. The mean fruit firmness was significantly affected by neither ethylene antagonist fumigation nor by storage type following 90 days of storage. The fruit fumigated with NC and 1-MCP maintained higher mean fruit firmness (54.95 N and 55.00 N, respectively) when compared to BC and control, following 120 days of storage (Table 6.2). The mean fruit firmness was significantly highest in the fruit stored in CA (55.24 N) when compared to ACA (52.35 N) storage following 120 days of storage (Table 6.2). There was no significant interaction effect between ethylene antagonist treatment and storage types on the fruit firmness values following both 90 and 120 days of storage.

Table 6.2. Effect of the ethylene antagonists and AiroFresh® on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Cripps Pink apple fruit stored in CA storage for 90 and 120 days

		Storage period (days)					
		90			120		
Parameters	Treatment	ACA	CA	Mean (T)	ACA	CA	Mean (T)
Physiological loss of weight (PLW)	Control	1.37±0.11	2.07±0.12	1.72	2.73±0.21	2.35±0.15	2.54B
	BC	1.36±0.12	1.61±0.09	1.49	2.26±0.04	1.64±0.22	1.95A
	NC	1.34±0.12	1.75±0.15	1.55	2.24±0.16	1.99±0.05	2.12A
	1-MCP	1.02±0.03	1.78±0.39	1.40	2.68±0.17	2.34±0.19	2.51B
	Mean (S)	1.27A	1.80B		2.48B	2.08A	
	LSD ($P \leq 0.05$)	T=ns	S=0.27	TXS=ns	T=0.35	S=0.25	TXS=ns
Fruit firmness	Control	54.08±0.80	53.43±1.05	53.76	51.10±0.48	53.71±1.20	52.41A
	BC	54.96±0.47	54.62±0.64	54.79	51.72±1.33	53.92±0.31	52.82AB
	NC	56.12±1.47	55.87±0.85	55.99	53.96±1.61	55.95±0.61	54.95B
	1-MCP	57.70±2.45	55.18±0.82	56.44	52.63±0.69	57.37±0.90	55.00B
	Mean (S)	55.72	54.78		52.35A	55.24B	
	LSD ($P \leq 0.05$)	T= ns	S=ns	TXS=ns	T=2.16	S=1.53	TXS=ns

ns=non-significant, T = treatments, S = storage conditions, n = 4 replicates (15 fruit (PLW) and 10 fruit (Firmness) per replication), Mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The upper case was used for treatment and storage period means. 90 and 120 days data were analysed separately.

6.3.2.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA

The mean SSC values were found to be significantly highest in fruit fumigated with BC (15.37 %) followed by NC and 1-MCP (15.15 % each) stored for 90 days. In case of the fruit stored for 120 days, the fruit fumigated with NC and 1-MCP exhibited significantly highest mean SSC values (15.15 % and 15.19 %) when compared to BC and control treatments (Table 6.3). The mean SSC values in the Cripps Pink fruit stored in ACA were significantly highest (15.19 %) following 90 days of storage (Table 6.3). The interaction effect between ethylene antagonist treatments and storage type on the SSC values following 120 days of storage life was significant. The control fruit stored in ACA exhibited the lowest SSC values when compared to all other treatments (Table 6.3).

The fruit fumigated with BC exhibited significantly highest mean TA (0.57 %) when compared to all other treatments following 90 days of storage (Table 6.3). There was no significant effect of ethylene antagonist treatments on the TA values following 120 days of storage. No significant effect of storage type was observed on the TA values in the fruit stored for both 90 and 120 days. There was a significant interaction effect between ethylene antagonist treatments and the storage type on the TA values following 120 days of storage. The fruit fumigated with NC and stored in CA for 120 days exhibited significantly lowest TA values when compared to all other treatments (Table 6.3).

The mean SSC: TA values were significantly lowest in the fruit fumigated with BC (26.87) when compared to all other treatments and stored for 90 days (Table 6.3). The storage type did not show any significant effect on SSC: TA values in the fruit stored for both 90 and 120 days. The interaction effect between ethylene antagonist treatment and storage type on SSC: TA values were significant in the fruit stored for 120 days (Table 6.3). The fruit treated with NC and stored in CA for 120 days showed significantly highest SSC: TA values (30.30) when compared to all other treatments (Table 6.3). There was no significant interaction effect between ethylene antagonist treatments and storage type in the 90 days stored fruit.

Table 6.3 Effect of the ethylene antagonists and AiroFresh® on the changes in the soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Cripps Pink apple fruit stored in CA storage for 90 and 120 days

		Storage period (days)					
		90			120		
Parameters	Treatment	ACA	CA	Mean (T)	ACA	CA	Mean (T)
Soluble solids concentration (SSC)	Control	14.72±0.02	14.60±0.08	14.66A	14.70±0.06a	15.05±0.06bc	14.88A
	BC	15.50±0.04	15.25±0.06	15.37C	15.03±0.09bc	14.80±0.04ab	14.91A
	NC	15.27±0.04	15.00±0.04	15.13B	15.10±0.04bc	15.20±0.05c	15.15B
	1-MCP	15.25±0.08	15.02±0.02	15.13B	15.05±0.06bc	15.33±0.18c	15.19B
	Mean (S)	15.19B	14.97A		14.97	15.09	
	LSD ($P \leq 0.05$)	T=0.12	S=0.09	TXS=ns	T=0.21	S=ns	TXS=0.30
Titratable acidity (TA)	Control	0.50±0.01	0.50±0.01	0.50A	0.55±0.01bcd	0.54±0.01bc	0.55
	BC	0.56±0.00	0.58±0.01	0.57B	0.53±0.02b	0.58±0.01e	0.56
	NC	0.51±0.02	0.51±0.01	0.50A	0.57±0.01de	0.50±0.01a	0.54
	1-MCP	0.51±0.01	0.49±0.01	0.51A	0.55±0.01bcd	0.56±0.00cde	0.56
	Mean (S)	0.52	0.52		0.55	0.55	
	LSD ($P \leq 0.05$)	T=0.03	S=ns	TXS=ns	T=ns	S=ns	TXS=0.03
SSC: TA	Control	29.35±0.56	29.10±0.65	29.23B	26.62±0.45abc	27.77±0.61bc	27.20
	BC	27.54±0.06	26.19±0.42	26.87A	28.34±1.07c	25.42±0.44a	26.88
	NC	29.93±0.95	30.49±0.46	30.21B	26.38±0.39ab	30.30±0.71d	28.34
	1-MCP	29.79±0.63	29.34±0.51	29.57B	27.26±0.53abc	27.23±0.32abc	27.25
	Mean (S)	29.15	28.78		27.15	27.68	
	LSD ($P \leq 0.05$)	T=1.47	S=ns	TXS=ns	T = ns	S = ns	TXS=1.71

ns=non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), Mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. 90 and 120 days data were analysed separately.

6.3.2.4 Individual sugars

The Cripps Pink apple fruit fumigated with NC exhibited significantly highest mean levels of glucose (21.40 g kg^{-1}), fructose (301.9 g kg^{-1}) and sorbitol (24.21 g kg^{-1}) but lowest mean sucrose levels (259.4 g kg^{-1}) when compared to all other treatments following 90 days of storage (Table 6.4). In case of the fruit stored for 120 days, the fruit fumigated with NC and 1-MCP exhibited significantly highest mean levels of glucose (17.82 g kg^{-1} and 21.69 g kg^{-1}, respectively) and fructose (235.2 g kg^{-1} and 238.7 g kg^{-1}, respectively) when compared to BC and control treatments (Tables 6.4). The mean levels of sucrose were significantly highest in the control fruit following both 90 days (293.80 g kg^{-1}) and 120 days (191.55 g kg^{-1}) (Table 6.4).

The fruit stored in CA for both 90 and 120 days exhibited significantly highest mean levels of glucose (13.3 g kg^{-1} and 19.72 g kg^{-1}, respectively) and sorbitol (22.53 g kg^{-1} and 19.05 g kg^{-1}) (Table 6.4). The mean levels of fructose were significantly highest in the fruit stored in CA for 120 days (Table 6.4). The storage type did not show any significant effect on the levels of sucrose following both 90 and 120 days of storage.

The interaction effect between the ethylene antagonist treatments and storage type on the mean levels of individual sugars (glucose, fructose, sucrose and sorbitol) was found to be significant. The fruit fumigated with NC and stored in ACA for 90 days exhibited highest glucose (23.10 g kg^{-1}), fructose (312.20 g kg^{-1}) and sorbitol (25.64 g kg^{-1}) levels and lowest sucrose (257.40 g kg^{-1}) levels (Table 6.4). The fruit fumigated with NC stored in ACA for 120 days showed the highest levels of glucose (28.78 g kg^{-1}) and lowest levels of sucrose (149.45 g kg^{-1}). The fruits fumigated with BC and stored in ACA for 120 days had lowest sorbitol levels (16.52 g kg^{-1}), while fruit treated with 1-MCP and stored in CA for 120 days exhibited highest fructose levels (262.40 g kg^{-1}) (Table 6.4).

Table 6.4 Effect of the ethylene antagonists and AiroFresh® on the levels of individual sugars (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in CA storage for 90 and 120 days

		Storage period (days)					
		90			120		
Sugars	Treatment	ACA	CA	Mean (T)	ACA	CA	Mean (T)
Glucose	Control	0.00±0.00a	4.20±3.64a	2.10A	0.00±0.00a	23.04±1.56de	11.52A
	BC	0.00±0.00a	14.70±4.63b	7.30AB	11.61±0.30bc	21.98±3.02de	16.79B
	NC	23.1±0.65b	19.60±1.32b	21.40C	28.78±0.88e	6.85±3.47b	17.82BC
	1-MCP	3.80±3.26a	14.90±4.32b	9.30B	16.36±1.16cd	27.02±0.69e	21.69C
	Mean (S)	6.72A	13.34B		14.19A	19.72B	
	LSD ($P \leq 0.05$)	T=5.92	S=4.18	TXS=8.37	T=4.61	S=3.26	TXS=6.52
Fructose	Control	275.30±4.48ab	265.70±2.92a	270.50A	202.50±4.96a	229.90±2.66c	216.20A
	BC	266.90±2.46a	289.50±5.80c	278.20A	201.70±2.62a	227.90±4.46c	214.80A
	NC	312.20±4.42d	291.60±2.36c	301.90C	225.30±1.25c	245.10±1.30d	235.20B
	1-MCP	293.30±2.71c	280.60±4.77bc	287.00B	215.00±3.37b	262.40±1.36e	238.70B
	Mean (S)	286.94	281.87		211.13A	241.34B	
	LSD ($P \leq 0.05$)	T=8.56	S=ns	TXS=12.11	T=6.23	S=4.40	TXS=8.80
Sucrose	Control	292.30±6.43	295.30±4.60	293.80C	214.56±2.03e	168.54±3.81bc	191.55B
	BC	286.40±4.52	277.20±3.87	281.80BC	175.17±1.94c	178.57±3.82c	176.87A
	NC	257.40±6.90	261.40±1.95	259.40A	149.45±2.49a	192.98±5.68d	171.21A
	1-MCP	274.00±4.08	268.60±7.23	271.30AB	178.37±1.12c	163.16±1.70b	170.77A
	Mean (S)	277.53	275.64		179.39	175.81	
	LSD ($P \leq 0.05$)	T=12.67	S=ns	TXS=ns	T=7.63	S=ns	TXS=10.78
Sorbitol	Control	21.00±0.52b	22.06±0.48bc	21.53B	19.73±0.75b	19.83±0.29b	19.78
	BC	17.21±0.36a	21.62±0.48b	19.41A	16.52±0.28a	19.71±1.48b	18.11
	NC	25.64±0.77d	22.78±0.53b	24.21C	19.58±0.62b	17.50±0.48ab	18.54
	1-MCP	21.60±0.56b	23.67±1.04bc	22.63B	16.63±0.60a	19.17±0.29b	17.90
	Mean (S)	21.36A	22.53B		18.11	19.05	
	LSD ($P \leq 0.05$)	T=1.26	S=0.89	TXS=1.79	T=ns	S=ns	TXS=2.31

ns=non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. 90 and 120 days data were analysed separately.

6.3.2.5 Individual organic acids

The reverse-phase HPLC used to quantify levels of individual sugars identified considerable amounts of malic acid and succinic acid, very low amounts of fumaric and citric acid but no tartaric acid in the pulp samples of Cripps Pink apple. The fruit treated with 1-MCP exhibited significantly highest mean levels of malic acid (6.72 g kg^{-1}) and succinic acid (0.71 g kg^{-1}) following 90 days of storage (Table 6.5). The fruit fumigated with NC and 1-MCP stored for 120 days showed significantly highest mean succinic acid (0.69 g kg^{-1} and 0.68 g kg^{-1}) levels (Table 6.5).

The fruit stored in ACA showed significantly lowest mean levels of malic acid (6.53 g kg^{-1}) in fruit stored for 120 days and succinic acid (0.61 g kg^{-1}) in fruit stored for 90 days (Table 6.5). The interaction effect between the ethylene antagonists and the storage types was significant for malic acid levels following 120 days of storage and succinic acid levels following 90 days of storage. The fruit fumigated with BC stored in ACA exhibited lowest levels of malic acid (5.32 g kg^{-1}) at 120 days of storage and succinic acid (0.50 g kg^{-1}) at 90 days of storage (Table 6.5).

6.3.2.6 Total phenols, ascorbic acid and total antioxidant capacity

The Cripps Pink apple fruit fumigated with NC exhibited significantly highest levels of total phenols following both 90 days (51.30 g GAE kg^{-1}) and 120 days (43.90 g GAE kg^{-1}) of storage (Table 6.6). There was no significant effect of storage type on the levels of total phenols following both the storage periods. No significant interaction effect was found between ethylene antagonist treatments and storage types in both 90 and 120 days of storage.

The ethylene antagonists, storage type and interaction between them did not show any significant effect on the levels of ascorbic acid following 90 and 120 days of storage. The fruit treated with ethylene antagonist (BC, NC and 1-MCP) exhibited higher mean levels of total antioxidant capacity following both 90 and 120 days of storage (Table 6.6). The levels of total antioxidant capacity were significantly highest (10.38 µM kg^{-1} Trolox) in the fruit stored in ACA following 90 days of storage, while levels were significantly lowest (9.80 µM kg^{-1} Trolox) in the fruit stored for 120 days in ACA (Table 6.6). There

was no significant interaction effect between ethylene antagonist treatments and storage types on levels of total antioxidant capacity following both 90 and 120 days of storage.

Table 6.5 Effect of the ethylene antagonists and AiroFresh® on the levels of individual organic acids (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in CA storage for 90 and 120 days

		Storage period (days)					
		90			120		
Organic acids	Treatment	ACA	CA	Mean (T)	ACA	CA	Mean (T)
Malic acid	Control	5.70±0.10	5.47±0.17	5.59B	6.01±0.25b	6.25±0.09bc	6.13
	BC	4.60±0.25	4.49±0.26	4.54A	5.32±0.24a	7.08±0.10d	6.20
	NC	5.56±0.18	5.31±0.32	5.43B	5.96±0.16b	6.75±0.05cd	6.35
	1-MCP	6.60±0.17	6.83±0.12	6.72C	6.13±0.20b	6.05±0.16b	6.09
	Mean (S)	5.61	5.53		5.85A	6.53B	
	LSD ($P \leq 0.05$)	T=0.348	S=ns	TXS=ns	T=ns	S=0.26	TXS=0.53
Succinic acid	Control	0.60±0.02bc	0.61±0.02bc	0.60C	0.57±0.02	0.60±0.02	0.58A
	BC	0.50±0.03a	0.50±0.03a	0.50A	0.61±0.02	0.58±0.0	0.59A
	NC	0.56±0.02b	0.56±0.02b	0.56B	0.68±0.01	0.70±0.01	0.69B
	1-MCP	0.65±0.01c	0.77±0.03d	0.71D	0.66±0.02	0.71±0.02	0.68B
	Mean (S)	0.58A	0.61B		0.63	0.64	
	LSD ($P \leq 0.05$)	T=0.04	S=0.03	TXS=0.05	T=0.04	S=ns	TXS=ns

ns=non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. 90 and 120 days data were analysed separately.

Table 6.6 Effect of the ethylene antagonists and AiroFresh® on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of the Cripps Pink apple fruit stored in CA storage for 90 and 120 days

		Storage period (days)					
		90			120		
	Treatment	ACA	CA	Mean (T)	ACA	CA	Mean (T)
Total phenols (g GAE kg^{-1})	Control	38.80±2.16	41.20±0.87	40.00A	34.30±1.81	41.90±0.78	38.10AB
	BC	37.40±7.79	38.50±2.32	38.00A	33.90±2.17	36.00±1.61	34.90A
	NC	46.80±4.25	55.80±4.51	51.30B	44.30±4.19	43.50±0.79	43.90B
	1-MCP	36.20±2.28	41.40±1.26	38.80A	34.50±1.77	37.70±4.98	36.10A
	Mean (S)	39.81	44.21		36.77	39.79	
	LSD ($P \leq 0.05$)	T=8.48	S=ns	TXS=ns	T=6.62	S=ns	TXS=ns
Ascorbic acid (g kg^{-1})	Control	9.63±0.24	9.67±0.36	9.65	12.89±0.27	13.52±0.10	13.20
	BC	9.86±0.30	9.72±0.22	9.79	12.31±0.09	12.66±0.23	12.48
	NC	10.56±0.22	10.37±0.19	10.46	13.01±0.53	12.87±0.13	12.94
	1-MCP	10.28±0.29	9.74±0.27	10.01	13.01±0.42	12.99±0.30	13.00
	Mean (S)	10.08	9.87		12.80	13.01	
	LSD ($P \leq 0.05$)	T=ns	S=ns	TXS=ns	T=ns	S=ns	TXS=ns
Total antioxidant capacity (µM kg^{-1} Trolox)	Control	9.71±0.25	9.03±0.11	9.37A	9.73±0.09	9.96±0.06	9.85
	BC	10.68±0.06	9.72±0.20	10.20B	9.72±0.17	10.29±0.43	10.00
	NC	10.69±0.17	10.55±0.18	10.62B	9.93±0.24	10.85±0.23	10.39
	1-MCP	10.43±0.09	10.12±0.53	10.27B	9.80±0.12	10.21±0.46	10.01
	Mean (S)	10.38B	9.86A		9.80A	10.33B	
	LSD ($P \leq 0.05$)	T= 0.54	S=0.3.82	TXS= ns	T=ns	S=0.42	TXS=ns

ns=non-significant, T = treatments, S = storage conditions, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant. The upper case was used for treatment and storage period means. 90 and 120 days data were analysed separately.

6.4 Discussion

In the present investigation, the effects of fumigation with new ethylene antagonists (BC and NC) and 1-MCP on the 'Cripps Pink' apple fruit stored in CA storage with and without the AiroFresh® technology were studied for the first time. The fumigation treatment with BC, NC and 1-MCP effectively delayed the ethylene and respiration climacteric peak onset as well as reduced the peak rates following both 90 and 120 days of storage. This demonstrates that the ethylene antagonists effectively bind to the ethylene receptors in the fruit and thus inhibit the ethylene action as well as autocatalytic ethylene production (Sisler, 2006). The ability of 1-substituted cyclopropenes to inhibit the ethylene action is by irreversibly attaching to ethylene receptors and further inhibition of expression of ethylene response genes as well as autocatalytic production of internal ethylene in the fruits (Sisler et al., 2003; Apelbaum et al., 2008). A similar mode of action for NC and BC was proposed by Musa (2016). The rates of ethylene and respiratory climacteric peaks were lower in the fruit stored in ACA following 90 days of storage, but following 120 days of storage, they were higher in the fruit stored in ACA. There is no work reported on the effects of AiroFresh® in combination with CA on postharvest fruit physiology during storage. The AiroFresh® manufacturers claim that the machine works on the principle of photocatalytic oxidation (PCO) (AiroFresh®, 2019). The maintenance of higher levels of O_2 concentrations is essential for effective and complete oxidation of ethylene by PCO in the closed storage rooms (Pathak et al., 2017). The levels of O_2 are maintained low in the CA storage (Gross et al., 2016) and there is a possibility of a reduction in the efficiency of AiroFresh® equipped in the CA storage, with extension in length of storage duration, due to lack of enough amounts of O_2. No significant interaction effect was recorded between the ethylene antagonist treatment and presence or absence of AiroFresh® in the CA storage on the respiration and ethylene production rates.

The PLW was lower in the 'Cripps Pink' fruit fumigated with BC, NC and 1-MCP when compared to control following both 90 and 120 days of storage. The loss of weight in the fruit during the storage is the result of physiological processes such as respiration and transpiration which in turn cause loss of water (Becker and Fricke, 1996). Similar to the trends of rates of ethylene and respiration, the PLW was also lower in the fruit stored in

ACA following 90 days of storage and higher in case 120 days stored fruit. The reduction in rates of ethylene production and respiration is associated with a decrease in loss of water from fruit and thus decline in the PLW in the fruits (Martínez-Romero et al., 2007).

The fruit firmness was maintained high in the fruit fumigated with BC, NC and 1-MCP in comparison to control, following 90 and 120 days of storage. The trend of fruit firmness was inverse to the trend of the PLW in the fruit stored in ACA following 90 and 120 days. The maintenance of higher fruit firmness could be associated with a reduction in rates of ethylene and respiration as well as a decrease in PLW during storage (Harker et al., 1997; Giovannoni, 2008). The fruit firmness during storage is reduced mainly due to loss of cell turgidity which is in turn due to loss of water (Van Buren, 1979). Softening of fruit during storage is due to the activity of cell wall hydrolyzing enzymes, which are in turn activated by the involvement of ethylene hormone (Giovannoni, 2008).

The levels of SSC were significantly higher in the BC, NC and 1-MCP treated apple fruit when compared the control fruit following 90 and 120 days of storage. The levels of SSC were higher in the apple fruit stored in ACA during 90 days of storage and lower following 120 days of storage. These trends depict that in the apple fruit the accumulation of SSC is not essentially associated with ethylene production and perception (Fan et al., 1999). The changes in levels of TA and SSC: TA due to ethylene antagonist treatments and by different storage environments were not significant or did not show a specific trend. Similarly, absence of distinctive trend or significant effect in the apple fruit treated with 1-MCP and stored in different storage environments was also reported by Mir et al. (2001) in Redchief Delicious apple and by Watkins et al. (2000) in McIntosh, Empire, Delicious and Law Rome apple fruits.

The levels of glucose, fructose and sorbitol are comparatively higher in the fruit treated with NC and 1-MCP following 90 and 120 days of storage. At the same time, the lowest levels of sucrose were recorded in fruit treated with NC and 1-MCP. These variations are due to the interconversion of glucose, fructose, sucrose and sorbitol with progress in the ripening process and also because of the variations in the storage environment (Ackermann et al., 1992). In general, the levels of sugars were higher in the apple fruit fumigated with ethylene antagonist when compared to control fruit. The similar trend of

higher levels of sugars in apple fruit treated with ethylene antagonist was also reported by Fan et al. (1999) through his experiments with 1-MCP on 'Redchief Delicious' apples.

The levels of total antioxidant capacity in the Cripps Pink fruit treated with ethylene antagonist (BC, NC and 1-MCP) were higher when compared to the control fruit. While the fruit fumigated with NC also exhibited the highest levels of total phenols. The process of fruit ripening includes several reactions which result in the production of significant amounts of ROS. The total phenols, ascorbic acids and flavonols form major constituents of bioactive compounds in the apple fruit and play a major role in degrading these ROS during the ripening process (Steinite et al., 2004; Łata and Tomala, 2007; Valero et al., 2016). Maintenance of higher levels of total phenols and total antioxidant capacity in the ethylene antagonist treated fruit attributed to the delay in the ripening process and a reduction in ethylene production rates (Masia, 1998).

6.5 Conclusions

The ethylene antagonists (BC, NC and 1-MCP) effectively reduced the rates of respiration and ethylene production as well as antagonised the ethylene-related physiological changes in the Cripps Pink apple fruit during both 90 and 120 days of controlled atmospheric storage. The installation of AiroFresh® technology in the controlled atmospheric storage exhibited a synergistic effect in reducing the ethylene and respiration rates as well as in maintaining the apple fruit quality following 90 days of storage. But this efficiency was reduced following 120 days when compared to conventional controlled atmospheric storage. Hence, storing the Cripps Pink apple fruit in the ACA for up to 90 days is ideal to derive the benefit of the synergistic effect of AiroFresh® and CA storage. The treatment of Cripps Pink apple fruit with NC showed results on par with 1-MCP, in lowering ethylene and respiration rates and in maintaining the fruit quality parameters such as higher levels of fruit firmness, total phenol and total antioxidant capacity as well as reducing PLW during controlled atmosphere storage with or without AiroFresh®. Hence the new ethylene antagonists have the capacity to be used as an alternative to 1-MCP to counteract the ethylene action in Cripps Pink fruit during CA storage. There was no significant interaction effect between ethylene antagonist treatment and type of CA

storage on the respiration and ethylene production rates as well as other parameters studied.

CHAPTER 7

Efficacy of postharvest fumigation with novel ethylene antagonists on ethylene production and fruit quality of Granny Smith and Cripps Pink apples stored in different controlled atmosphere storages

Abstract

The fruit ripening process is the last stage of fruit development and involves several irreversible physical, physiological and biochemical modifications in fruit leading to senescence and deterioration. The plant hormone ethylene directly or indirectly promotes these changes associated with the fruit ripening process. Regulating the ripening process by modifying the storage conditions and by antagonising the action of ethylene can significantly extend the storage life while maintaining the fruit quality and reduce postharvest losses. In the present research, the effect of two new ethylene antagonists, 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) and 1-methylcyclopropene (1-MCP) on the rates of respiration and ethylene production as well as on the postharvest fruit quality were studied in the Granny Smith apple stored in CA storage for 90 and 120 days as well as in the Cripps Pink apple stored in CA storage equipped with AiroFresh® technology for 60 and 150 days. The fruit were fumigated with ethylene antagonists in 60 L hermetically sealed plastic drums for 18 h before transferring them into respective storage rooms. The untreated fruit were considered as the control. The experiment was laid in completely randomized design and each treatment was replicated four times. All the ethylene antagonist treatments (BC, NC and 1-MCP) effectively reduced the rates of respiration and ethylene production as well as maintained lower PLW and higher fruit firmness, total phenol and ascorbic acid in both Granny Smith and Cripps Pink apple fruit at the different storage environments and durations studied. Comparatively, the fumigation treatment with 1-MCP was most efficient in reducing rates of ethylene and respiration climacteric peaks. But the BC and NC exhibited results on par with 1-MCP in maintaining most of the fruit quality parameters studied.

7.1 Introduction

The process of fruit ripening is considered as the final stage of the fruit development and involves several catabolic and anabolic reactions, which lead to senescence and deterioration of the fruit. The fruit undergoes several modifications during the ripening process and typically involves changes in respiration and ethylene production trends, degradation of cell wall materials, conversion of starch to sugars, the formation of aroma volatiles and depletion of bioactive compounds (Tucker, 2012).

The phytohormone ethylene is naturally produced by almost all the plant parts and is directly or indirectly responsible for several irreversible physical, physiological and biochemical changes in plant parts (Arteca, 2013). The role of ethylene in promoting the fruit ripening has been well demonstrated and it is estimated to be responsible for up to 50 % of all the postharvest losses in fruit and vegetables (Blanke, 2014). The apple (*Malus*× *domestica* Borkh.) fruit is classified as 'climacteric fruit' as it exhibits a sharp rise in rates of respiration and ethylene during ripening process (Giovannoni, 2008). The apple fruit is highly sensitive to ethylene exposure and also produce large amounts of ethylene gas during the ripening process. The ripening-associated modifications promoted by ethylene significantly affect the postharvest quality and storage life of the fruits (Abeles et al., 1992). Several methods are being adopted to manage the ethylene in order to increase the storage life, reduce postharvest losses and maintain fruit quality during storage (Gross et al., 2016). Treating fruits with 1-substituted cyclopropenes (CP) is considered as a most effective method to counteract the negative effects of ethylene by irreversibly blocking the ethylene receptors and interfering with the expression of ethylene-responsive genes (Watkins, 2006; Apelbaum et al., 2008). The 1-methylcyclopropene (1-MCP) is recognised as relatively stable CP at the gaseous state and the commercial formulations are widely being used to extend the storage life of different horticulture produce (Valero et al., 2016).

The controlled atmosphere (CA) consists of a storage environment with higher levels of CO_2 and low O_2 levels when compared to the normal atmosphere. The temperatures in the CA storage are maintained lowest possible and relative humidity high optimum to specific fruit crop. CA storage conditions significantly lower the rates of respiration and ethylene

production and ultimately extend the storage life of fruits stored in it (Keller et al., 2013). The apple fruit stored in CA storage tends to exhibit a sudden increase in the levels of ethylene production after removing from the storage. Application of ethylene antagonists along with CA helps to prevent this sudden rise in ethylene production (Watkins, 2006; Bai et al., 2009). AiroFresh® is a relatively new technology developed by Creative Research Technology (CRT), South Australia, Australia. The technology claims to degrade ethylene by advanced oxidation processes (AOP) and photocatalytic oxidation (PCO) as well as induce favourable conditions to enhance the efficiency of ethylene antagonists in the closed storage environments (AiroFresh®, 2019).

The positive effects of 1-MCP treatment in regulating the ethylene effects in the fruits and vegetables have been reported by several researchers yet it still has some limitations. The 1-MCP boils at temperatures as low as 0°C and vaporises immediately under normal atmospheric conditions (Sisler et al., 2006). The 1-MCP is available as expensive service and not affordable to all the fruit growers. There exists a necessity to explore the alternative compounds which could block ethylene action as effectively as 1-MCP and also address the limitations of 1-MCP. Considering these points two new ethylene antagonist compounds BC and NC were developed by Singh et al. (2018). In the present experiments, the effectiveness of the BC, NC and 1-MCP in antagonising the ethylene action was investigated in the Granny Smith apple fruit stored in CA storage and in the Cripps Pink apple fruit stored in CA storage with AiroFresh® at two different storage durations. It was hypothesised that all the ethylene antagonists will effectively antagonise the ethylene action, delay ripening-associated changes and maintain fruit quality in different storage environments and durations studied.

7.2 Material and methods

7.2.1 Fruit and experimental conditions

7.2.1.1 Experiment 1: Effects of new ethylene antagonist treatments on ethylene production and postharvest quality of Granny Smith apple fruit stored in controlled atmospheric storage

The Granny Smith apple fruit used in the experiment were obtained from Manjimup (34°13' S latitude, 116°08' E longitude) at commercial harvest (fruit firmness 60.95 ± 3.21 N; SSC 10.33 ± 0.80 %; TA 0.79 ± 0.02 %) during 13th April 2017. The Granny Smith trees were 11-years old and grafted on M26 rootstock trained as modified central leader. The trees were planted at a spacing of 4.5 m (within rows) and 1 m (between rows) with North-South orientation. The harvested fruit were dipped in an aqueous solution mixture of 'Scholar' (a.i. 230 g L^{-1} fludioxonil), 'Caltop' (a.i. 165 g L^{-1} calcium), 'DPA' (diphenylamine) to prevent postharvest diseases and disorders during the storage. The fruit were then air-dried properly and packed in corrugated cardboard boxes with soft board trays to prevent any bruises or mechanical injuries. The packed fruit were then immediately transported using the air-conditioned vehicle to the Curtin Horticulture Research Laboratory, Perth.

The apple fruit with a relatively uniform size which were free from bruises, mechanical injuries, pests or diseases were selected for the experiment. The selected fruit were fumigated with ethylene antagonists (BC, NC and 1-MCP) for 18 h in the 60 L hermetically sealable plastic drums, keeping sixty fruit in each drum. The fumigation procedure for ethylene antagonists were detailed in Chapter 3, Section 3.3.1. The apple fruit without any treatment were considered as control. The experiment was laid in a completely randomised design with each treatment replicated four times and keeping fifteen fruit in each treatment. After completion of the fumigation treatment, the plastic drums were opened in the open-air environment and the fruits were packed in corrugated cardboard boxes with softboard trays with respect to the treatments. Sixty fruits (four replications and fifteen fruit per replication) were arranged in one box and the boxes were labelled accordingly. All the boxes were divided into two lots, each lot meant to be stored

in different CA storages at grower's property in Manjimup for 90 and 120 days. The gas concentrations in the CA were maintained at 2.5 ± 0.64 % O_2 and 1.3 ± 0.45 % CO_2 and temperature at 0 ± 1 °C. The storage durations and the CA storage environment were determined according to the commercial storage practices of the fruit growers. On completion of the designated CA storage period the fruit were immediately brought back to the laboratory to analyse physiological and fruit quality parameters.

7.2.1.2 Experiment 2: Effects of fumigation with novel ethylene antagonists on the postharvest physiology and quality of Cripps Pink apple fruit stored in controlled atmospheric storage with AiroFresh®

The Cripps Pink apple fruit used for the experiment were sourced from Balingup (34°13' S latitude, 116°08' E longitude) during 5th May 2018 while commercial harvest stage (fruit firmness 66.76±3.84 N; SSC 15.03±0.04 %; TA 0.82±0.04 %). The Cripps Pink apple trees were 23-year old and grafted on MM106 rootstock. The trees were planted at 4.5 m (within rows) and 3 m (between rows) spacing with North-South orientation and trained as modified central leader. The harvested fruit were dipped in an aqueous solution of 'Magnate 750WG' (a.i. 750 g L^{-1} Imazalil) @ 0.68 g L^{-1}, 'Stopit' (a.i. 160 g L^{-1} liquid calcium chloride ($CaCl_2$) @ 15 mL L^{-1} and DPA (diphenylamine) @ 5 mL L^{-1} to protect from postharvest diseases and disorders during storage. The fruit were then placed in soft board trays and packed in corrugated cardboard boxes after air-drying them properly in open air. The air-conditioned vehicle was used to transport them to Curtin Horticulture Research Laboratory, Perth.

For the experiment, the apple fruit of relatively uniform size without any bruises, mechanical injuries, pest or disease symptoms were chosen. The chosen fruit were fumigated with ethylene antagonists (BC, NC and 1-MCP) as explained in Chapter 3, Section 3.3.1, keeping sixty fruit per drum. The experiment was laid in a completely randomised design with four replications and fifteen fruit per replication. The treated fruit were packed in corrugated cardboard boxes with respect to the treatments, labelled adequately and immediately transferred to CA storages at grower's property at Kirup, WA (33°42' S latitude, 115°53' E longitude). The CA storage was equipped with AiroFresh®

technology and the gas concentrations were maintained at 3.45 ± 0.45 % O_2 and 2.40 ± 0.36 % CO_2 and temperature at 0 ± 1 °C. The CA storage conditions and the length of storage period were decided based upon the commercial storage practices of the fruit growers as well as according to the availability of the commercial storage facilities. On completion of 60 and 150 days of the storage period, the fruit were brought back to the laboratory using the air-conditioned vehicle for analysis of physiological and fruit quality parameters.

7.2.2 Determination of fruit physiological parameters

Two fruit per experimental unit were randomly chosen to determine the rates of respiration and ethylene production.

7.2.2.1 Rate of ethylene production

The two selected fruit were incubated in the airtight 1 L glass jar for 1 h and sample (1 mL) of headspace gas drawn from individual glass jars was injected into GC (6890N Network GC system, Agilent Technology, CA, USA) to determine the amount of ethylene produced. The detailed description of instrument and procedure are explained in Chapter 3, Section 3.5.1. The rate of ethylene production was determined daily till distinctive climacteric peak was achieved. The rate of ethylene production calculated is expressed as µmol C_2H_4 kg^{-1} h^{-1}.

7.2.2.2 Rate of respiration

The sample (2 mL) of headspace gas was drawn from 1 L glass jar with two selected fruit, hermetically sealed for 1 h. The gas sample is injected into Infrared gas analyser (Servomex Gas Analyser, 1450 Food Package Analyser, Servomex Limited, UK) to estimate the rate of respiration based upon the carbon dioxide levels. The detailed description of machine and calculations to estimate the rate of respiration are mentioned in Chapter 3, Section 3.5.2. The respiration rate calculated is expressed as mmol CO_2 kg^{-1} h^{-1}.

7.2.3 Determination of fruit quality parameters

7.2.3.1 Physiological loss of weight (PLW)

The initial weight of fifteen fruit per experimental unit was recorded before transferring them into respective storage rooms. The final fruit weight was recorded after completion of designated storage period. The formula to calculate PLW from initial and final weights is mentioned in Chapter 3, Section 3.6.1. The calculated PLW is expressed as %.

7.2.3.2 Fruit firmness

The fruit firmness is calculated using Texture Analyser (TA Plus, Ametek Lloyd Instruments Limited, UK) from ten randomly chosen fruit per experimental unit. The detailed procedure and calculations to determine fruit firmness is mentioned in Chapter 3, Section 3.6.2. The fruit firmness determined is expressed in newtons (N).

7.2.3.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA

The infrared digital refractometer (Atago- Palette PR 101, Atago Co., Tokyo, Japan) was used to determine SSC from the juice samples and expressed as % Brix. The titration method was used to estimate TA and expressed as % malic acid. The detailed procedure and calculations for SSC, TA and SSC: TA are explained in Chapter 3, Section 3.6.3.

7.2.3.4 Individual sugars and organic acids

The levels of individual sugars (glucose, sucrose, fructose and sorbitol) and individual organic acids (malic acid, succinic acid, fumaric acid, tartaric acid and citric acid) were estimated using reverse-phase HPLC system (Waters 1525, Milford Corporation, USA) from the pooled pulp samples extracted from thirteen fruit per replication. The Dual λ UV absorbance detector (Water 2487, Milford Corporation, USA) at 214 nm was used to quantify individual organic acids, while Refractive Index (RI) detector (Water 2414, Milford Corporation, USA) was used for the individual sugars. The description of instruments and procedures are mentioned in detail in Chapter 3, Section 3.6.5. The levels of sugars and organic acids determined are expressed as g kg^{-1}.

7.2.3.5 Total phenols

The levels of total phenols in the fruit pulp samples were determined by Folin-Ciocalteu reagent method following the procedures detailed in Chapter 3, Section 3.6.6. The standard curve drawn from pure gallic acid was used to calculate total phenolic content and is expressed as g GAE kg^{-1} fresh weight basis.

7.2.3.6 Ascorbic acid

The ascorbic acid in the fruit pulp samples were determined using UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK) following the procedure detailed in Chapter 3, Section 3.6.7. The standard curve drawn using pure L-ascorbic acid was used to calculate ascorbic acid levels and expressed as g kg^{-1} fresh weight basis.

7.2.3.7 Total antioxidant capacity

The levels of total antioxidant capacity in the fruit pulp samples were determined using DPPH method following the procedure explained in Chapter 3, Section 3.6.8. The curve drawn from standard Trolox was used to calculate levels of total antioxidant capacity and expressed as µM kg^{-1} Trolox fresh weight basis.

7.2.4 Statistical analysis

The data recorded for all the observations was statistically analysed using *GenStat* software 14.0 version (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The results were tabulated as means ± standard errors (SE) of the means. The respiration and ethylene production data were analysed by one-way and data of all other parameters were analysed through two-way analysis of variance (ANOVA). The least significant difference (LSD) was determined using the F-test with 5% error probability. Duncan's multiple comparison tests were used to compare treatment means.

7.3 Results

7.3.1 Fruit physiological parameters

7.3.1.1 Ethylene (C_2H_4) production

The fumigation treatment with ethylene antagonists (BC, NC and 1-MCP) have significantly ($P \leq 0.05$) delayed the C_2H_4 climacteric peak onset when compared to control fruit in both Granny Smith and Cripps Pink apple fruit (Table 7.1 and 7.2, Figure 7.1) during respective CA storage conditions. The mean C_2H_4 climacteric peak onset was delayed for the significantly longest time in the Granny Smith fruit fumigated with 1-MCP following both 90 and 120 days of CA storage (13.25 and 10.00 days, respectively), followed by the fruit fumigated with NC and BC (Table 7.1). Even in case of the Cripps Pink fruit, the fumigation treatment with 1-MCP has delayed the C_2H_4 climacteric peak onset for the longest time during both 60 and 150 days of storage (12.50 and 8.75 days, respectively) followed by the fruit fumigated with BC and NC (Table 7.2).

The mean C_2H_4 climacteric peak rates were significantly lower in the fruit fumigated with BC, NC and 1-MCP in both Granny Smith and Cripps Pink apple fruits (Table 7.1 and 7.2, Figure 7.1). The Granny Smith apple fruit fumigated with 1-MCP exhibited lowest C_2H_4 climacteric peak rates for both 90 and 120 days of CA storage followed by NC and BC treatments (Table 7.1). Similarly, in the Cripps Pink fruit, the 1-MCP treatment significantly reduced the mean C_2H_4 climacteric peak rates to the lowest during both 60 and 150 days of storage (0.03 and 0.04 µmol $kg^{-1}h^{-1}$, respectively) followed by BC and NC treatments in sequence (Table 7.2).

7.3.1.2 Respiration (CO_2 production)

The effect of the ethylene antagonist treatments on the respiratory climacteric peak onset was not significant in both Granny Smith and Cripps Pink apple fruit following the respective storage periods. The rates of the respiratory climacteric peak were lower in the Granny Smith and Cripps Pink apple fruit fumigated with BC, NC and 1-MCP when compared to the control fruit (Table 7.1 and 7.2). The fruit fumigated with 1-MCP exhibited significantly lowest mean respiratory peak rates in Granny Smith (0.55 mmol $kg^{-1}h^{-1}$) following 120 days and Cripps Pink (0.50 mmol $kg^{-1}h^{-1}$) following 150 days of storage (Table 7.1 and 7.2).

Table 7.1 Effect of the ethylene antagonists on the climacteric peak onset (days) and peak rate of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) of Granny Smith apple fruit stored in CA storage.

	Storage period (days)			
Treatment	90		120	
	C_2H_4 climacteric peak onset	C_2H_4 climacteric peak rate	C_2H_4 climacteric peak onset	C_2H_4 climacteric peak rate
Control	8.75±1.08a	2.26±0.07c	2.00±0.00a	3.88±0.12d
BC	9.50±0.75a	1.85±0.13c	6.25±0.65b	2.11±0.02c
NC	11.25±1.29ab	0.62±0.17b	6.25±0.65b	0.82±0.06b
1-MCP	13.25±0.65b	0.01±0.00a	10.00±0.00c	0.00±0.00a
LSD ($P \leq 0.05$)	3.11	0.44	1.39	0.25
	Respiration climacteric peak onset	Respiration climacteric peak rate	Respiration climacteric peak onset	Respiration climacteric peak rate
Control	5.75±1.24	1.25±0.12	4.50±0.25	1.05±0.09c
BC	6.75±1.08	1.12±0.04	5.50±0.90	0.85±0.03b
NC	8.25±1.52	0.83±0.03	6.50±1.15	0.68±0.05ab
1-MCP	9.75±2.01	0.96±0.11	5.00±0.50	0.55±0.03a
LSD ($P \leq 0.05$)	ns	ns	ns	0.18

ns = non-significant, n = 4 replicates (2 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns. Mean values followed by a similar letter are not significantly different within the columns. Mean values without letters within columns are non-significant. 90 and 120 days data were analysed separately.

Table 7.2 Effect of the ethylene antagonists on the climacteric peak onset (days) and peak rate of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) of Cripps Pink apple fruit stored in CA storage with AiroFresh®.

	Storage period (days)			
Treatment	60		150	
	C_2H_4 climacteric peak onset	C_2H_4 climacteric peak rate	C_2H_4 climacteric peak onset	C_2H_4 climacteric peak rate
Control	8.00±0.87a	1.78±0.06b	3.25±0.22a	3.59±0.25c
BC	12.00±0.87bc	1.48±0.15b	6.75±0.89b	2.00±0.41b
NC	9.00±0.00ab	1.57±0.07b	6.75±0.89b	2.41±1.02b
1-MCP	12.50±2.17c	0.03±0.03a	8.75±1.08b	0.04±1.06a
LSD ($P \leq 0.05$)	3.30	0.36	2.54	0.86
	Respiration climacteric peak onset	Respiration climacteric peak rate	Respiration climacteric peak onset	Respiration climacteric peak rate
Control	8.75±0.22	0.66±0.03	4.50±0.36	0.74±0.04b
BC	9.75±0.74	0.62±0.04	8.25±0.09	0.68±0.02b
NC	10.00±1.70	0.61±0.03	7.25±0.19	0.65±0.04b
1-MCP	11.00±0.87	0.55±0.01	6.00±0.03	0.50±0.03a
LSD ($P \leq 0.05$)	ns	ns	ns	0.14

ns = non-significant, n = 4 replicates (2 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns. Mean values followed by a similar letter are not significantly different within the columns. Mean values without letters within columns are non-significant. 60 and 150 days data were analysed separately.

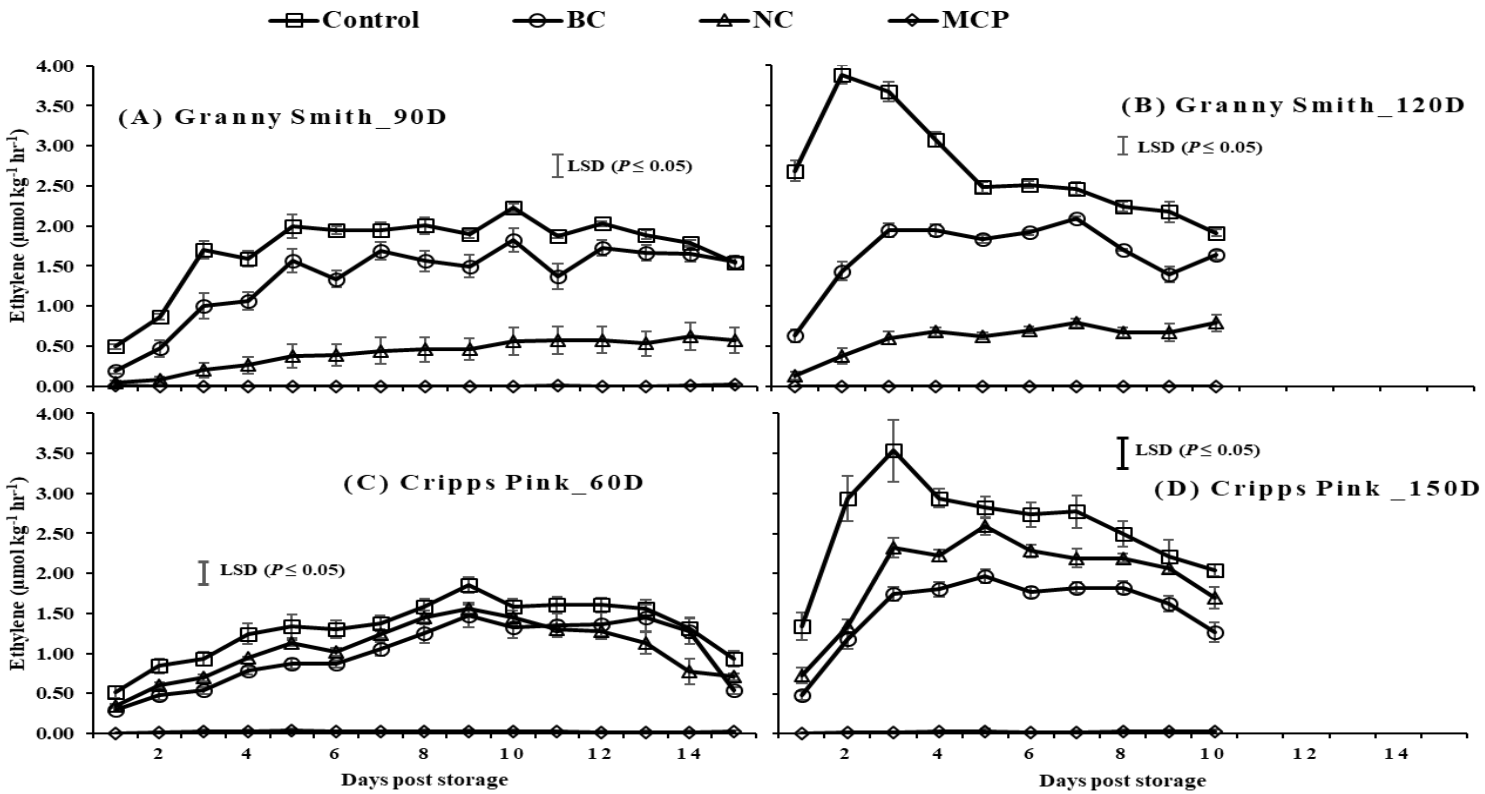


Figure 7.1 Changes in the rate of ethylene production due to the ethylene antagonist treatment (T) days post storage (D) in Granny Smith apple fruit stored in CA storage for 90 and 120 days and in Cripps Pink apple fruit stored in CA storage with AiroFresh® for 60 and 150 days. The vertical bars represent SE of mean values and are not visible when values are smaller than the symbol. n=4 replicates (2 fruit per replication). LSD ($P \leq 0.05$) (A) T=0.08, D=0.14, TXD=0.28 (B) T=0.07, D=0.11, TXD=0.22 (C) T=0.07, D=0.14, TXD=0.28 (D) T=0.12, D=0.19, TXD=0.39.

7.3.2 Fruit quality parameters

7.3.2.1 Physiological loss of weight (PLW)

The Granny Smith and Cripps Pink apple fruit fumigated with BC, NC and 1-MCP exhibited lower mean PLW when compared to control fruit (Table 7.3 and 7.4). The Granny Smith fruit fumigated with NC exhibited comparatively lowest mean PLW (1.46 %) followed by BC (1.50 %) and 1-MCP (1.60 %) (Table 7.3). In the case of Cripps Pink, the fruit fumigated with BC and 1-MCP showed the lowest mean PLW (3.20 % each) (Table 7.4). The mean PLW increased with the extension of storage duration in both Granny Smith (from 1.57 to 1.71 %) and Cripps Pink (2.31 to 4.37 %) apples (Table 7.3 and 7.4). There was no significant interaction effect between ethylene antagonist treatments and storage duration on the PLW in both Granny Smith and Cripps Pink apple fruits.

7.3.2.2 Fruit firmness

The mean fruit firmness in the Granny Smith and Cripps Pink apple fruit fumigated with BC, NC and 1-MCP was significantly higher when compared to control fruit (Table 7.3 and 7.4). The fruit fumigated 1-MCP exhibited significantly highest mean fruit firmness in both Granny Smith (52.27 N) and Cripps Pink (56.23 N) followed by the fruit treated with BC and NC (Table 7.3 and 7.4). The mean fruit firmness reduced with the extension of storage life in both Granny Smith (from 49.24 to 48.73 N) and Cripps Pink (55.42 to 50.66 N) apple fruit (Table 7.3 and 7.4). There was a significant interaction effect between ethylene antagonist treatments and storage duration on the fruit firmness in both the apple cultivars studied. The Granny Smith fruit treated with 1-MCP stored for 90 days exhibited significantly highest fruit firmness (52.53 N), while the Cripps Pink fruit fumigated with 1-MCP stored for 60 days exhibited significantly highest fruit firmness (57.27 N) (Table 7.3 and 7.4).

Table 7.3 Effect of the ethylene antagonists on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Granny Smith apple fruit stored in CA storage.

	Storage period (days)		
Treatment	90	120	Mean (T)
	Physiological loss of weight (PLW)		
Control	1.94±0.10	2.07±0.11	2.01 B
BC	1.49±0.11	1.51±0.10	1.50 A
NC	1.34±0.09	1.59±0.08	1.46 A
1-MCP	1.52±0.09	1.67±0.11	1.60 A
Mean (D)	1.57	1.71	
LSD ($P \leq 0.05$)	T=0.22	D=ns	TXD=ns
	Fruit firmness		
Control	46.33±0.31 b	43.77±0.60 a	45.05 A
BC	48.62±0.47 c	47.96±0.22 bc	48.29 B
NC	49.48±0.31 de	51.17±0.89 de	50.33 C
1-MCP	52.53±0.56 e	52.02±0.59 e	52.27 D
Mean (D)	49.24	48.73	
LSD ($P \leq 0.05$)	T=1.32	D=ns	TXD=1.86

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (15 fruit (PLW) and 10 fruit (fruit firmness) per replication), mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

Table 7.4 Effect of the ethylene antagonists on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Cripps Pink apple fruit stored in CA storage with AiroFresh®.

	Storage period (days)		
Treatment	60	150	Mean (T)
	Physiological loss of weight (PLW)		
Control	2.76±0.22	4.54±0.25	3.65
BC	2.02±0.49	4.38±0.12	3.20
NC	2.33±0.21	4.32±0.17	3.32
1-MCP	2.15±0.35	4.24±0.16	3.20
Mean (D)	2.31A	4.37B	
LSD ($P \leq 0.05$)	T=ns	D=0.45	TXD=ns
	Fruit firmness		
Control	53.88±1.08bc	46.48±0.55a	50.18A
BC	54.63±1.56bc	52.56±1.01a	53.60B
NC	55.89±0.38bc	48.42±1.05a	52.16AB
1-MCP	57.27±0.87c	55.19±0.57bc	56.23C
Mean (D)	55.42B	50.66A	
LSD ($P \leq 0.05$)	T=2.29	D=1.62	TXD=3.24

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (15 fruit (PLW) and 10 fruit (fruit firmness) per replication), mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

7.3.2.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA

The Granny Smith fruit fumigated with ethylene antagonists (BC, NC and 1-MCP) exhibited significantly higher mean SSC values when compared to the control fruit (11.93 %) (Table 7.5). Whilst, the Cripps Pink fruit fumigated with BC (16.21 %) and 1-MCP (16.20 %) showed significantly lower mean SSC values when compared to control (16.51 %) and fruit fumigated with NC (16.47 %) (Table 7.6). The storage duration did not show any significant effect on the mean SSC values in both Granny Smith and Cripps Pink fruit. There was a significant interaction effect recorded between ethylene antagonist treatments and the storage duration on the SSC values in both the cultivars studied. The Granny Smith apple fumigated with NC and stored for 90 days (13.38 %) as well as the Cripps Pink fruit fumigated with NC and stored for 60 days (16.63 %) exhibited significantly highest SSC values when compared to all other treatments (Tables 7.5 and 7.6).

The mean TA value of the control fruit was significantly highest in the Granny Smith apple (0.75 %), while the Cripps Pink control fruit had lowest TA values (0.61 %) when compared to all the ethylene antagonist treatments (Tables 7.5 and 7.6). The mean TA values reduced significantly with the extension of the storage period in both Granny Smith (from 0.77 to 0.63 %) and Cripps Pink (0.66 to 0.60 %) (Table 7.5 and 7.6). The interaction effect between ethylene antagonists and storage duration on the TA values was significant in Granny Smith but non-significant in Cripps Pink apple fruit. The Granny Smith fruit fumigated with NC and control fruit stored for 90 days (0.82 % each) exhibited significantly highest TA values when compared to all other treatments (Table 7.5).

The mean SSC: TA values of the Granny Smith control fruit was significantly lowest (16.16) but the Cripps Pink control fruit exhibited significantly highest SSC: TA values (27.34) when compared to all the ethylene antagonist treatments (Table 7.5 and 7.6). The mean SSC: TA values increased significantly with the extension of storage duration in both Granny Smith (from 16.46 to 20.25) and Cripps Pink (24.76 to 27.23) apple fruit (Tables 7.5 and 7.6). The interaction effect between ethylene antagonist treatments and the storage duration on the SSC: TA values was significant for both Granny Smith and

Cripps Pink fruit. The Granny Smith fruit treated with NC and stored for 120 days exhibited significantly highest SSC: TA values when compared to all other treatments (Table 7.5). Whilst, the Cripps Pink control fruit stored for 150 days showed significantly highest SSC: TA values (Table 7.6).

Table 7.5 Effect of the ethylene antagonists on the changes in the soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Granny Smith apple fruit stored in CA storage.

	Storage period (days)		
Treatment	90	120	Mean (T)
	Soluble solids concentration (SSC)		
Control	11.58±0.07 a	12.28±0.02 b	11.93 A
BC	12.48±0.35 b	12.50±0.00 b	12.49 B
NC	13.38±0.17 d	13.20±0.14 cd	13.29 C
1-MCP	13.20±0.38 cd	12.65±0.06 bc	12.93 C
Mean (D)	12.66	12.66	
LSD ($P \leq 0.05$)	T=0.43	D=ns	TXD=0.61
	Titratable acidity (TA)		
Control	0.82±0.01 d	0.67±0.02 bc	0.75 B
BC	0.72±0.02 c	0.63±0.01 ab	0.68 A
NC	0.82±0.02 d	0.58±0.01 a	0.70 A
1-MCP	0.72±0.02 c	0.62±0.01 ab	0.67 A
Mean (D)	0.77B	0.63A	
LSD ($P \leq 0.05$)	T=0.04	D=0.03	TXD=0.06
	SSC: TA		
Control	14.06±0.25 a	18.27±0.43 c	16.16A
BC	17.26±0.28 bc	19.75±0.28 d	18.51B
NC	16.26±0.29 b	22.66±0.38 e	19.46C
1-MCP	18.26±0.21 c	20.32±0.34 d	19.29BC
Mean (D)	16.46 A	20.25 B	
LSD ($P \leq 0.05$)	T=0.79	D=0.56	TXD=1.11

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

Table 7.6 Effect of the ethylene antagonists on the changes in the soluble solid concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Cripps Pink apple fruit stored in CA storage with AiroFresh®.

	Storage period (days)		
Treatment	60	150	Mean (T)
	Soluble solids concentration (SSC)		
Control	16.60±0.06e	16.43±0.02d	16.51B
BC	16.00±0.04a	16.43±0.02d	16.21A
NC	16.63±0.02e	16.33±0.02cd	16.47B
1-MCP	16.15±0.04b	16.25±0.04bc	16.20A
Mean (D)	16.34	16.36	
LSD ($P \leq 0.05$)	T=0.09	D=ns	TXD=0.13
	Titratable acidity (TA)		
Control	0.65±0.01	0.56±0.01	0.61A
BC	0.65±0.01	0.59±0.01	0.62AB
NC	0.65±0.01	0.62±0.01	0.64BC
1-MCP	0.68±0.00	0.63±0.01	0.66C
Mean (D)	0.66B	0.60A	
LSD ($P \leq 0.05$)	T=0.02	D=0.02	TXD=ns
	SSC: TA		
Control	25.42±0.24bc	29.26±0.78e	27.34C
BC	24.51±0.28ab	27.73±0.46d	26.12B
NC	25.47±0.34bc	26.23±0.44c	25.85B
1-MCP	23.63±0.06a	25.69±0.44bc	24.66A
Mean (D)	24.76A	27.23B	
LSD ($P \leq 0.05$)	T=1.04	D=0.73	TXD=1.47

ns= non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

7.3.2.4 Individual sugars

The Granny Smith apples fumigated with BC exhibited significantly lowest mean levels of fructose (330.02 g kg^{-1}), sucrose (79.53 g kg^{-1}) and sorbitol (14.38 g kg^{-1}) when compared to all other treatments (Table 7.7). The ethylene antagonist treatments did not show any significant effect on the mean levels of glucose in the Granny Smith apples. Similarly, the ethylene antagonist treatments did not show any significant effect on the

mean levels of fructose, sucrose and sorbitol in the Cripps Pink apple fruit. The Cripps Pink apple fruit treated with 1-MCP exhibited significantly highest amounts of glucose (12.55 g kg^{-1}) when compared to remaining treatments (Table 7.8).

There was a significant reduction in the mean levels of fructose (from 361.78 to 342.74 g kg^{-1}), sucrose (from 110.70 to 342.74 g kg^{-1}) and sorbitol (from 16.93 to 14.80 g kg^{-1}) with the extension of storage duration from 90 to 120 days in the Granny Smith apple fruit but there was no significant effect of storage duration on the mean levels of glucose. In the Cripps Pink apple fruit, a significant increase in the mean levels of glucose (from 10.35 to 342.74 g kg^{-1}) and fructose (from 162.28 to 177.34 g kg^{-1}) were recorded with the extension of storage duration from 60 to 150 days (Table 7.8). The mean levels of sucrose and sorbitol were not significantly affected by the extension of storage duration in Cripps Pink apple fruit.

There was a significant interaction effect between ethylene antagonist treatments and the storage duration on the levels of glucose, fructose, sucrose and sorbitol in Granny Smith apple fruit. The Granny Smith fruit fumigated with 1-MCP and stored for 90 days exhibited highest levels of glucose (231.40 g kg^{-1}) and fructose (380.31 g kg^{-1}) when compared to all other treatments (Table 7.7). Whilst, significantly highest levels of sucrose (123.66 g kg^{-1}) and sorbitol (19.33 g kg^{-1}) were recorded in the Granny Smith fruit fumigated with NC and stored for 90 days (Table 7.7). In case of the Cripps Pink apple fruit, the interaction effect between ethylene antagonists and the storage duration was significant for the mean levels of glucose, fructose and sucrose but non-significant for sorbitol levels. The fruit fumigated with 1-MCP and stored for 150 days exhibited significantly highest levels of glucose (14.48 g kg^{-1}) and fructose (182.00 g kg^{-1}) compared to all other treatments (Table 7.8). The Cripps Pink fruit fumigated with NC and stored for 150 days exhibited significantly highest levels of sucrose (230.04 g kg^{-1}) in comparison with all other treatments (Table 7.8).

Table 7.7 Effect of the ethylene antagonists on the levels of individual sugars (g kg^{-1}) in the pulp of the Granny Smith apple fruit stored in CA storage.

	Storage period (days)		
Treatment	90	120	Mean (T)
	Glucose		
Control	158.70±6.45 a	201.00±6.56 b	179.85
BC	197.93±2.86 b	180.61±9.85 ab	189.27
NC	196.40±4.44 b	198.67±1.76 b	197.54
1-MCP	231.40±19.48 c	176.46±3.57 ab	203.93
Mean (D)	196.11	189.19	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=28.88
	Fructose		
Control	344.41±8.96 bc	363.15±3.73 cd	353.78 B
BC	344.93±4.98 bc	321.11±4.40 a	333.02 A
NC	377.45±5.51 d	350.37±1.22 bc	363.91 B
1-MCP	380.31±8.42 d	336.32±5.05 ab	358.32 B
Mean (D)	361.78 B	342.74 A	
LSD ($P \leq 0.05$)	T=13.25	D=9.37	TXD=18.75
	Sucrose		
Control	110.32±3.0 c	83.18±4.54 b	96.74 C
BC	103.79±7.46 c	55.27±5.38 a	79.53 A
NC	123.67±1.92 d	60.12±1.37 a	91.89 BC
1-MCP	105.02±2.78 c	63.68±2.68 a	84.35 AB
Mean (D)	110.70 B	65.56 A	
LSD ($P \leq 0.05$)	T=9.31	D=6.58	TXD=13.17
	Sorbitol		
Control	15.09±0.66 ab	16.48±0.42 bc	15.79 AB
BC	16.18±1.10 b	12.58±0.49 a	14.38 A
NC	19.33±0.41 c	15.18±0.12 ab	17.25 B
1-MCP	17.12±1.79 bc	14.95±0.51 ab	16.04 AB
Mean (D)	16.93 B	14.80 A	
LSD ($P \leq 0.05$)	T=1.94	D=1.37	TXD=2.74

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

Table 7.8 Effect of the ethylene antagonists on the levels of individual sugars (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in CA storage with AiroFresh®.

	Storage period (days)		
Treatment	60	150	Mean (T)
	Glucose		
Control	9.80±0.65b	12.00±0.10c	10.90B
BC	10.58±1.00bc	10.76±0.22bc	10.67B
NC	10.40±0.40bc	7.92±0.26a	9.16A
1-MCP	10.62±0.31bc	14.48±0.81d	12.55C
Mean (D)	10.35A	11.29B	
LSD ($P \leq 0.05$)	T=1.09	D=0.77	TXD=1.54
	Fructose		
Control	158.52±3.43a	183.93±1.09d	171.23
BC	166.92±3.16abc	170.65±3.18bc	168.79
NC	162.83±3.17abc	172.80±4.00cd	167.81
1-MCP	160.86±1.79ab	182.00±3.12d	171.43
Mean (D)	162.28A	177.34B	
LSD ($P \leq 0.05$)	T=ns	D=5.38	TXD=10.77
	Sucrose		
Control	209.56±5.19ab	225.63±2.14bc	217.60
BC	211.69±3.92ab	212.61±3.20ab	212.15
NC	214.53±5.35abc	230.04±6.46c	222.28
1-MCP	221.74±2.69bc	198.14±4.11a	209.94
Mean (D)	214.38	216.61	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=14.87
	Sorbitol		
Control	18.37±0.36	20.59±0.29	19.48
BC	20.91±0.52	20.50±0.68	20.70
NC	20.86±0.63	21.75±1.10	21.31
1-MCP	20.92±0.40	20.84±0.36	20.88
Mean (D)	20.27	20.92	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

7.3.2.5 Individual organic acids

The RP-HPLC systems quantified significant levels of malic acid, very low levels of succinic acid, tartaric acid and fumaric acid but no citric acid in the apple fruit samples studied. The levels of individual acids identified were higher in the fruit fumigated with ethylene antagonists (BC, NC and 1-MCP) when compared to the control fruit in both Granny Smith and Cripps Pink apples. The Granny Smith apple fruit fumigated with 1-MCP exhibited significantly highest mean levels of malic acid (5.27 g kg^{-1}) and succinic acid (0.76 g kg^{-1}) followed by the fruit treated with NC and BC (Table 7.9). Similarly, the Cripps Pink fruit fumigated with 1-MCP exhibited significantly highest amounts of malic acid (7.88 g kg^{-1}) when compared to all other treatments (Table 7.10).

With the extension of storage period from 90 to 120 days an increased level of malic acid (from 4.39 to 5.29 g kg^{-1}) and succinic acid (0.58 to 0.73 g kg^{-1}) were recorded in Granny Smith apple fruit (Table 7.9). There was no significant interaction effect between ethylene antagonists and the storage duration on the levels of individual organic acids in both Granny Smith and Cripps Pink apple fruit.

Table 7.9 Effect of the ethylene antagonists on the levels of individual organic acids (g kg^{-1}) in the pulp of the Granny Smith apple fruit stored in CA storage.

	Storage period (days)		
Treatment	90	120	Mean (T)
	Malic acid		
Control	3.65±0.20	5.10±0.29	4.38 A
BC	4.22±0.31	5.23±0.24	4.73 AB
NC	4.79±0.08	5.17±0.08	4.98 BC
1-MCP	4.87±0.27	5.66±0.20	5.27 C
Mean (D)	4.39A	5.29B	
LSD ($P \leq 0.05$)	T=0.50	D=0.34	TXD=ns
	Succinic acid		
Control	0.49±0.01	0.64±0.02	0.57 A
BC	0.56±0.03	0.68±0.03	0.62 B
NC	0.60±0.01	0.77±0.01	0.69 C
1-MCP	0.69±0.02	0.82±0.02	0.76 D
Mean (D)	0.58 A	0.73 B	
LSD ($P \leq 0.05$)	T=0.05	D=0.03	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant.

Table 7.10 Effect of the ethylene antagonists on the levels of malic acid (g kg^{-1}) in the pulp of the Cripps Pink apple fruit stored in CA storage with AiroFresh®.

	Storage period (days)		
Treatment	60	150	Mean (T)
	Malic acid		
Control	6.39±0.18	6.41±0.33	6.40A
BC	7.69±0.15	6.91±0.16	7.30AB
NC	6.56±1.01	6.55±0.32	6.56A
1-MCP	8.28±0.20	7.48±0.26	7.88B
Mean (D)	7.23	6.84	
LSD ($P \leq 0.05$)	T=0.98	D=ns	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant.

7.3.2.6 Total phenols

The mean total phenol levels in the Granny Smith and Cripps Pink apple fruit fumigated with 1-MCP was significantly highest (85.70 and 25.07 g GAE kg^{-1}, respectively) when compared to all other treatments (Table 7.11 and 7.12). With the extension of the storage duration the levels of total phenols significantly reduced in both Granny Smith (from 82.45 to 65.49 g GAE kg^{-1}) and Cripps Pink (24.51 to 19.41 g GAE kg^{-1}) (Table 7.11 and 7.12). The interaction effect between the ethylene antagonists and storage duration was significant in both Granny Smith and Cripps Pink apple fruits. The Granny Smith apple fruit fumigated with BC and stored for 90 days exhibited the highest total phenol levels when compared to all other treatments (90.85 g GAE kg^{-1}) (Table 7.11). Whilst. the Cripps Pink fruit fumigated with 1-MCP and stored for 60 days showed significantly highest total phenol levels (30.41 g GAE kg^{-1}) (Table 7.12).

7.3.2.7 Ascorbic acid

The Granny Smith apple fruit fumigated with BC, NC and 1-MCP showed significantly higher mean ascorbic acid levels when compared to control fruit. The fruit fumigated with BC exhibited the highest ascorbic acid levels (14.16 g kg^{-1}) (Table 7.11). In case of Cripps Pink apple fruit, the mean ascorbic acid level was highest in the fruit fumigated with 1-MCP (12.47 g kg^{-1}) followed by NC treatment (11.98 g kg^{-1}) (Table 7.12). The levels of ascorbic acid significantly increased with the extension of storage duration from 90 to 120 days in Granny Smith apple (from 12.51 to 14.08 g kg^{-1}) but there was no significant effect of storage duration in Cripps Pink apple fruit (Table 7.11 and 7.12). The interaction effect between ethylene antagonist treatment and storage duration on ascorbic acid levels was significant in Granny Smith apple but it was non-significant in Cripps Pink apple fruit. The Granny Smith apple fruit fumigated with BC and stored for 120 days exhibited significantly highest ascorbic acid content (15.90 g kg^{-1}) when compared to all other treatments (Table 7.11).

7.3.2.8 Total antioxidant capacity

The Granny Smith apple fruit fumigated with BC showed the significantly highest level of total antioxidant capacity (17.63 µM kg^{-1} Trolox) when compared to all other

treatments (Table 7.11). There was no significant effect of ethylene antagonist treatment on the level of total antioxidant capacity in Cripps Pink apple fruit. The effect of storage duration on total antioxidant capacity levels was non-significant in both Granny Smith and Cripps Pink apple fruit. The Granny Smith apple fruit fumigated with BC and stored for 120 days exhibited significantly highest total antioxidant capacity when compared to all other treatments (Table 7.11). There was no significant interaction effect between ethylene antagonist treatment and storage duration on total antioxidant activity levels in Cripps Pink fruit.

Table 7.11 Effect of the ethylene antagonists on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of the Granny Smith apple fruit stored in CA storage.

	Storage period (days)		
Treatment	90	120	Mean (T)
	Total phenols (g GAE kg^{-1})		
Control	62.13±3.70 b	49.87±0.48 a	56.00 A
BC	90.85±1.81 c	64.47±5.19 b	77.66 B
NC	86.90±1.18 c	66.15±1.88 b	76.52 B
1-MCP	89.91±2.16 c	81.50±2.59 c	85.70 C
Mean (D)	82.45 B	65.49 A	
LSD ($P \leq 0.05$)	T=6.70	D=4.74	TXD=9.48
	Ascorbic acid (g kg^{-1})		
Control	12.48±0.48 a	12.08±0.33 a	12.28 A
BC	12.41±0.23 a	15.90±0.25 b	14.16 B
NC	12.69±0.29 a	14.04±0.46 b	13.37 B
1-MCP	12.45±0.09 a	14.30±0.58 b	13.38 B
Mean (D)	12.51 A	14.08 B	
LSD ($P \leq 0.05$)	T=0.94	D=0.66	TXD=1.33
	Total antioxidant capacity (µM kg^{-1} Trolox)		
Control	15.50±0.40 a	15.64±0.63 a	15.57 A
BC	16.39±0.56 a	18.88±0.81 b	17.63 B
NC	16.93±0.38 ab	14.82±0.40 a	15.88 A
1-MCP	15.68±0.92 a	15.00±0.70 a	15.34 A
Mean (D)	16.13	16.08	
LSD ($P \leq 0.05$)	T=1.42	D=ns	TXD=2.01

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

Table 7.12 Effect of the ethylene antagonists on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of the Cripps Pink apple fruit stored in CA storage with AiroFresh®.

Storage period (days)			
Treatment	60	150	Mean (T)
Total phenols (g GAE kg^{-1})			
Control	23.02±1.34ab	19.18±1.51a	21.10A
BC	20.12±0.70ab	18.99±1.44a	19.55A
NC	24.51±1.44b	19.74±0.83ab	22.13AB
1-MCP	30.41±1.64c	19.74±1.40ab	25.07B
Mean (D)	24.51B	19.41A	
LSD ($P \leq 0.05$)	T=3.12	D=2.21	TXD=4.41
Ascorbic acid (g kg^{-1})			
Control	11.66±0.16	11.61±0.24	11.63AB
BC	11.00±0.17	11.33±0.35	11.16A
NC	12.64±0.68	11.31±0.05	11.98AB
1-MCP	12.33±0.34	12.62±0.43	12.47B
Mean (D)	11.91	11.72	
LSD ($P \leq 0.05$)	T=0.86	D=ns	TXD=ns
Total antioxidant capacity (µM kg^{-1} Trolox)			
Control	10.26±0.65	10.61±0.57	10.44
BC	9.57±0.10	10.25±0.38	9.91
NC	10.37±0.22	10.71±0.32	10.54
1-MCP	10.32±0.32	11.00±0.33	10.66
Mean (D)	10.13	10.65	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

7.4 Discussion

The effects of new ethylene antagonists (BC and NC) and 1-MCP on the postharvest physiology and fruit quality of Granny Smith apple stored in CA storage and Cripps Pink apple stored in CA storage along with AiroFresh® technology were investigated for the first time. The fumigation treatment with BC, NC and 1-MCP delayed the onset of ethylene climacteric peak and reduced the peak rate of respiratory and ethylene

climacteric peaks when compared to control in both Granny Smith and Cripps Pink apple fruits during CA storage conditions studied. This suggests that the all the ethylene antagonist treatments (BC, NC and 1-MCP) were effective in retarding internal ethylene production and inhibiting ethylene action in the Granny Smith and Cripps Pink apple fruit (Sisler, 2006). The ethylene antagonistic effect of 1-MCP is attributed to the ability of 1-alkyl cyclopropenes to bind with ethylene receptor sites in the fruit irreversibly and inhibit expression of the ethylene response genes (Sisler et al., 2003; Apelbaum et al., 2008). Musa (2016) has proposed a similar mode of action of BC and NC for ethylene antagonistic activity. The phytohormone ethylene directly or indirectly is responsible for several physical, physiological and biochemical changes associated with fruit ripening and thus critically affect the postharvest quality of the fruit during storage (Imaseki, 1991; Anwar et al., 2018).

The Granny Smith and Cripps Pink apple fruit fumigated with ethylene antagonists (BC, NC and 1-MCP) exhibited significantly reduced PLW when compared to control fruit during storage. Becker and Fricke (1996) have explained that the weight loss in the fruit during the storage is mainly due to loss of water as a result of different physiological processes such as respiration and transpiration. Hence reduced PLW in ethylene antagonist treated fruit could be related to the decreased rates of respiration and ethylene production (Martínez-Romero et al., 2007). The PLW in Granny Smith and Cripps Pink apple fruit increased significantly with the extension of storage life. The rise of PLW is due to the continuous respiration process carried out by the fruit during storage, therefore the longer the storage life the higher PLW is in fruit (Maguire et al., 2001).

The fruit firmness of the apple fruit is one of the important deciding character for consumer preference and hence its retention during storage is essential (DeEll et al., 1999). The Granny Smith and Cripps Pink apple fruit fumigated with BC, NC and 1-MCP significantly retained higher fruit firmness than control throughout the storage period. Van Buren (1979) and Giovannoni (2008) detailed that the loss of fruit firmness during storage is mainly due to loss of cell turgidity caused by water loss and by the fruit softening caused by cell wall hydrolysing enzymes. Hence the retention of fruit firmness in ethylene antagonist treated fruit could be associated with a reduction in PLW in the

fruit (Harker et al., 1997; Giovannoni, 2008). The activity of fruit softening enzymes is triggered mainly by the presence of ethylene hormone. Thus, the retention in fruit firmness could also be related to a reduction in ethylene production in the apple fruit fumigated with ethylene antagonists (Giovannoni, 2008).

The levels of SSC were significantly higher in the Granny Smith apple fruit treated with ethylene antagonist compounds while in case of Cripps Pink fruit fumigated with ethylene antagonist the SSC levels were lower when compared to control. Blankenship and Dole (2003) also reported contrasting effects of ethylene antagonist treatments with different apple cultivars and suggested that the effects of ethylene antagonists on the fruit quality are specific to different cultivars. This trend also suggest that accumulation of SSC in apple fruit during storage is not necessarily affected by the ethylene (Fan et al., 1999). The levels of TA were significantly lower in the Granny Smith fruit fumigated with ethylene antagonists, but they were significantly higher in case of Cripps Pink apple fruit treated with ethylene antagonists. The similar variations with different apple varieties were also reported by Watkins et al. (2000) when McIntosh, Empire, Delicious and Law Rome apple cultivars were treated with 1-MCP and stored under different storage conditions.

The higher levels of glucose, fructose and sorbitol and lower sucrose levels were recorded in the Granny Smith fruit fumigated with 1-MCP and NC when compared to control and BC treatment. Similarly, even in case of the Cripps Pink apple fruit fumigated with 1-MCP had higher levels of glucose, fructose and sorbitol and lower levels of sucrose when compared to other treatments. The variations among the levels of different individual sugars are due to interconversion of individual sugars during the ripening process and also due to variations in the storage conditions (Ackermann et al., 1992). Fan et al. (1999) also recorded the higher levels of sugars in the apple fruit fumigated with ethylene antagonist during his experiments with 1-MCP treatment on 'Redchief Delicious' apples. The levels of individual organic acids identified were higher in the fruit fumigated with ethylene antagonist when compared to control fruit, in both Granny Smith and Cripps Pink apple fruit. The organic acids are continuously broken down to form sugars to be used as respiratory substrates during the fruit ripening process. The maintenance of higher

levels of organic acids during storage in the fruit fumigated with ethylene antagonist can be attributed to a reduction in rates of respiration and ethylene production by the treatments (Giovannoni, 2008).

The levels of the total phenols and ascorbic acids were higher in the fruit treated with ethylene antagonist when compared to control in both Granny Smith and Cripps Pink apple fruits. The total antioxidant activity was higher in the Granny Smith fruit fumigated with ethylene antagonist treatments. The fruit ripening is an oxidative process and involves the production of significant amounts of reactive oxygen species (ROS). The bioactive compounds in the fruit are actively involved in the degradation of ROS formed and get depleted with the progress of ripening process (Masia, 1998; Valero et al., 2016). The total phenols, ascorbic acids and flavonols form major constituents of bioactive compounds in the apple fruit (Łata and Tomala, 2007). Retention of higher levels of total phenols and ascorbic acids during storage depicts delay in the ripening process and can be related to the reduction in rates of ethylene production and respiration in fruit treated with ethylene antagonists (Masia, 1999; Steinite et al., 2004; Valero et al., 2016).

7.5 Conclusions

The fumigation treatment with ethylene antagonists (BC, NC and 1-MCP) effectively delayed the ethylene climacteric peak and retarded rates of respiration and ethylene production in Granny Smith and Cripps Pink fruit during storage. The ethylene antagonists performed effectively in 90 and 120 days of CA stored Granny Smith apples as well as in the Cripps Pink apple fruit stored for 60 and 150 days in CA storage installed with AiroFresh®. The treatment with BC, NC and 1-MCP maintained lower PLW and higher fruit firmness, total phenols and ascorbic acid during storage when compared to control in both the cultivars studied. Comparatively, 1-MCP treatment suppressed ethylene and respiration in a more effective way than NC and BC. In case of the other fruit quality parameters studied, the fruit treated with BC and NC exhibited results on par with that of 1-MCP treatment. The new ethylene antagonist compounds possess the capability to be used as an alternative to 1-MCP. But studying the effects of different concentrations of NC and BC in antagonizing ethylene action warrants further investigation to effectively reduce ethylene and respiration similar to that of 1-MCP.

CHAPTER 8

Efficacy of postharvest fumigation of novel ethylene antagonists in regulating ethylene production and fruit quality of Gold Rush pears during controlled atmosphere storage

Abstract

The phytohormone ethylene is responsible for various irreversible changes associated with the fruit ripening process. The ripening process involves changes in the trends of respiration and ethylene production, fruit softening, physiological loss of weight and depletion of bioactive compounds in the fruits. In the present study, the effectiveness of two new compounds that antagonise ethylene, namely 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) as well as 1-methylcyclopropene (1-MCP) in retarding rates of respiration and ethylene production as well as in maintaining fruit quality of Gold Rush pear during 150 and 200 days of CA storage were investigated. The Gold Rush pear fruit were fumigated with ethylene antagonists for 18 h using 60 L hermetically sealable plastic drums and the untreated fruit were considered as the control. The experiments were laid in one or two-factor factorial completely randomised design with four replicates and fifteen fruit per replication. In comparison with the control fruit, the fumigation treatment with BC, NC and 1-MCP significantly ($P \leq 0.05$) reduced respiration and ethylene climacteric peak rates following both 150 and 200 days of CA storage. The pear fruit fumigated with ethylene antagonists (BC, NC and 1-MCP) maintained significantly higher mean fruit firmness and lower physiological loss of weight (PLW) than the control fruit. The fruit fumigated with BC and NC exhibited lower mean values of SSC, glucose and sorbitol compared to other treatments. The levels of organic acids, total phenols, ascorbic acid and total antioxidant capacity were not

This chapter has been presented at "2019 ASHS Annual Conference | July 21 - 25, 2019 | Las Vegas, NV, USA": **Vijay Yadav Tokala**, Zora Singh, Alan D. Payne and Poe Nandar Kyaw. 2019. New ethylene antagonists regulate ethylene action and maintain fruit quality in long term-controlled atmosphere stored Gold Rush pear.

significantly affected by any of the treatments. In conclusion, 1-MCP followed by BC and NC retarded ethylene and respiration rates and the new ethylene antagonist compounds (BC and NC) were equally effective in maintaining the postharvest fruit quality of Gold Rush pears stored at CA storage.

8.1 Introduction

Gold Rush pear (*Pyrus communis* L.) have an appearance similar to that of Beurre Bosc pears. They are dark brown in colour, sensitive to ethylene and ripen to form soft and buttery fruit flesh. Pear fruits are categorised as climacteric fruit based upon the trends of the rate of respiration and ethylene production during the ripening process. In general, most of the European pears need a period of chilling to initiate and continue normal ripening (Villalobos-Acuña and Mitcham, 2008). The fruit ripening is a complex process involving several catabolic and anabolic reactions, which ultimately cause senescence and deterioration in fruit. It is considered as an irreversible process and typically involves changes in levels of ethylene production and respiration rates, hydrolysis of cell wall components, fruit softening, development of aroma volatiles and depletion of bioactive compounds such as ascorbic acid and phenolic compounds (Tucker, 2012).

The plant hormone ethylene is produced in almost all the plant parts and is responsible for different physical, physiological and biochemical changes associated with fruit ripening (Anwar et al., 2018). The exposure to external ethylene gas accelerates the ripening process in climacteric fruits by further initiating autocatalytic internal ethylene production (Imaseki, 1991). Appropriate postharvest handling and optimum storage conditions can significantly extend storage life while maintaining the commercially acceptable quality of pear fruit (Gross et al., 2016).

In the CA storage, the fruit are stored in high CO_2 and low O_2 storage environment in addition to the lowest safe temperatures and high relative humidity. The rate of postharvest physiological activities are significantly reduced with a reduction in storage temperatures, thus extends storage life and maintain fruit quality for longer time (Kitinoja, 2013). Manipulating the gas concentrations in the CA storage environment

extends the storage life of the fruit to a considerable extent by lowering rates of respiration, ethylene and other changes associated with ripening process (Keller et al., 2013). Fumigation with 1-alkylcyclopropenes counteracts the negative effects of ethylene in the fruits by blocking ethylene receptor sites and interfering with the expression of ethylene response genes (Watkins, 2006; Apelbaum et al., 2008). 1-MCP in the gaseous state is recognised as relatively stable cyclopropene and widely being used in the commercial horticulture to prolong the storage life of different fruit and vegetables (Valero et al., 2016). The storing 1-MCP treated pear fruit in CA storage enables long-term storage of fruit while maintaining consumer acceptable fruit quality when compared to untreated fruit stored in CA (Bai et al., 2009). The effectiveness of the combined application of CA and 1-MCP depends upon the cultivar used, stage of maturity at harvest, and storage temperatures (Bai et al., 2009). The pome fruits stored in the CA storage tend to show a distinctive rise in ethylene production after removing from storage. Application of ethylene antagonists along with CA is found to retard this sudden rise in ethylene concentrations (Watkins, 2006; Bai et al., 2009).

Several researchers have recorded positive effects of 1-MCP in regulating ethylene action in fruit and vegetables (Valero et al., 2016). The boiling point of 1-MCP is as low as 0°C, which makes it unstable at room temperatures and vaporises immediately (Sisler et al., 2006). It is not easily available to the growers, expensive and is usually available as a service rather than as a compound. Keeping these points in view, the following experiment was designed to investigate the effects of fumigation treatment with two newly developed ethylene antagonists (BC and NC) on the ethylene production, respiration and other quality parameters in the Gold Rush pear fruit stored in CA storage. It was hypothesised that the all the ethylene antagonists will effectively antagonise ethylene action and maintain optimum fruit quality in the Gold Rush pear following both 150 and 200 days of CA storage.

8.2 Material and methods

8.2.1 Fruit and experimental conditions

The Gold Rush pear used as experiment fruit were collected at commercial harvest from Beedelup, WA (34°19' S latitude, 116°00' E longitude) at commercial maturity stage (fruit firmness 82.03 ± 4.14 N; SSC 11.43 ± 0.05 %; TA 0.09 ± 0.01 %) during 20th March 2018. The fruit were harvested from 19 years old Gold Rush trees grafted on *Pyrus calleryana* D6 (Callery Pear) rootstock and planted in North-South orientation with a spacing of 4 m (within rows) and 2 m (between rows). The trees were trained in modified central leader system. The harvested fruit were dipped in an aqueous solution of 'Magnate 750WG' (a.i. 750g L^{-1} Imazalil) @ 0.68 g L^{-1}, DPA (diphenylamine) 1.6 mL L^{-1} and 'Rovral' (a.i. 250 g L^{-1} Iprodiene) @ 1 mL L^{-1} in order to protect them from postharvest disorders or diseases during CA storage. Following the fungicidal dip, all the fruit were air-dried till there are no water droplets left on fruit surface and then placed in softboard trays before packing them in corrugated cardboard boxes. The packed fruit were then transported immediately to Curtin Horticulture Research Laboratory, Perth using the air-conditioned vehicle.

The fruit of relatively uniform size, free from mechanical injuries, bruises, pests or diseases were selected for the experiment purpose. The selected fruit were arranged in the hermetically sealable drums at a rate of sixty fruit per drum and fumigated with ethylene antagonists (BC, NC and 1-MCP) as detailed in Chapter 3, Section 3.3.1. On completion of fumigation treatment, the fruit were taken out of the drum in the open-air and were arranged on the softboard trays and packed in corrugated cardboard boxes. The experiment was laid in a completely randomised block design and each treatment replicated four times with fifteen fruit per replication. The boxes were labelled appropriately according to the treatments. The untreated fruit were considered as control. The packed boxes were divided into two lots, each lot meant for storage in two separate commercial CA storage rooms for 150 and 200 days. The fruit were then immediately transferred to commercial CA storage at Carmel, WA (32°00' S latitude 116°06' E longitude) with gas concentrations maintained at 2.3 ± 0.5 % O_2 and 0.4 ± 0.15 % CO_2 and 0.5 ± 0.71 °C. The storage time was decided to suit the growers' requirements. The fruit were taken out of the CA storage on completion of designated storage periods. The

fruit were brought back to Curtin Horticulture Research Laboratory immediately, using the air-conditioned vehicle to analyse physiological and quality parameters.

8.2.2 Determination of fruit physiological parameters

On completion of the designated storage period in the CA storage, two fruit per replication (experimental unit) were randomly chosen for determination of the rate of ethylene production and respiration.

8.2.2.1 Rate of ethylene production

The two selected fruit were placed in 1 L hermetically sealed glass jar for one hour at room temperature (20 ± 2 °C). A 1 mL gas sample was drawn from the headspace of the individual incubated jars through the rubber septum and injected into GC (6890N Network GC system; Agilent Technology, CA, USA) to determine the amount of the ethylene produced by the sealed pear fruit. The rate of ethylene production was determined daily until the distinct climacteric peak was achieved. The procedure and calculations for estimation of the ethylene produced are explained in detail in Chapter 3, Section 3.5.1. The rate of ethylene production calculated is expressed as µmol C_2H_4 kg^{-1} h^{-1}.

8.2.2.2 Rate of respiration

The selected two pear fruit were enclosed in a 1 L air-tight glass jar for one hour at room temperature (at 20±2°C). A 2 mL gas sample drawn from the headspace of individual glass jars was injected into Infrared gas analyser (Servomex Gas Analyser, 1450 Food Package Analyser, Servomex Limited, UK) to determine levels of carbon dioxide. The respiration rate in the fruit was estimated based upon the levels of carbon dioxide evolved and the detailed calculations are explained in Chapter 3, Section 3.5.2. The calculated rate of respiration in the pear fruit is expressed as mmol CO_2 kg^{-1} h^{-1}.

8.2.3 Determination of fruit quality parameters

8.2.3.1 Physiological loss of weight (PLW)

Fifteen fruit per replication were weighed before transferring fruit into the assigned CA storage and recorded as initial weight. On completion of designated CA storage period, the fruit were weighed again to record as final weight. The PLW was calculated using the formula as detailed in Chapter 3, Section 3.6.1. The calculated PLW of pear fruit is expressed as %.

8.2.3.2 Fruit firmness

Ten fruit per each replication were randomly selected after completion of the designated CA storage period to determine the fruit firmness. The Texture Analyser (TA Plus, Ametek Lloyd Instruments Limited, UK) was used to determine fruit firmness following the procedure and calculations detailed in Chapter 3, Section 3.6.2. The calculated fruit firmness is expressed in newtons (N).

8.2.3.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA

The pooled juice sample extracted from the slices cut from thirteen fruit per replication was used to determine SSC, TA and SSC: TA. The SSC was determined using infrared digital refractometer (Atago- Palette PR 101, Atago Co., Tokyo, Japan) and expressed as % Brix. The TA of the juice samples was determined using the titration method and expressed as % malic acid. The SSC: TA is determined by diving the SSC values by TA values. The detailed procedures and calculations for SSC and TA were mentioned in Chapter 3, Section 3.6.3.

8.2.3.4 Individual sugars and organic acids

The levels of individual sugars (glucose, fructose, sucrose and sorbitol) and individual acids (malic acid, succinic acid, fumaric acid, tartaric acid and citric acid) were estimated using reverse-phase HPLC system (Waters 1525, Milford Corporation, USA). The details about the preparation of samples and running them on HPLC system as well as the calculations were explained in Chapter 3, Section 3.6.5. The Dual λ UV absorbance detector (Water 2487, Milford Corporation, USA) at 214 nm was used to determine levels of individual organic acids, while levels of individual sugars were estimated using

Refractive Index (RI) detector (Water 2414, Milford Corporation, USA). The calculated levels of sugar and organic acids are expressed as g kg^{-1}.

8.2.3.5 Total phenols

The levels of total phenols in the pulp samples were determined using Folin-Ciocalteu reagent method and UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK). The details about the sample preparation, estimation procedure and calculations were mentioned in Chapter 3, Section 3.6.6. The standard curve drawn using pure gallic acid was used to calculate the total phenol levels and are expressed as g GAE kg^{-1} fresh weight basis.

8.2.3.6 Ascorbic acid

The levels of ascorbic acid were estimated using the UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK). The detailed explanation of sample preparation, procedure and calculations were mentioned in Chapter 3, Section 3.6.7. The standard curve drawn using pure L-ascorbic acid was used as a reference to calculate ascorbic acid levels and is expressed as g kg^{-1} fresh weight basis.

8.2.3.7 Total antioxidant capacity

The levels of the total antioxidant capacity in the fruit pulp were estimated using DPPH method outlined by Brand-Williams et al. (1995) with some modifications as explained by Vithana et al. (2018). The details of sample preparation, procedure and calculations were mentioned in Chapter 3, Section 3.6.8. The calculated values of total antioxidant capacity are expressed as µM kg^{-1} Trolox fresh weight basis.

8.2.4 Statistical analysis

The data of different parameters obtained in the experiment was statistically analysed by using *GenStat* software version 14.0 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The results were tabulated and presented as means ± standard errors (SE) of the means. The data of fruit physiological parameters were analysed by

one-way analysis and two-way analysis of variance (ANOVA) was used to analyse the data of all other parameters. The least significance difference (LSD) was determined using F-test with 5 % error probability. Duncan multiple comparison tests were used to comparing the significant difference between the mean values.

8.3 Results

8.3.1 Fruit physiological parameters

8.3.1.1 Ethylene (C_2H_4) production

The climacteric peak rates were significantly ($P \leq 0.05$) reduced in the Gold Rush pear fruit fumigated with ethylene antagonists (BC, NC and 1-MCP) when compared to control fruit, following both 150 and 200 days of CA storage (Figure 8.1). The control fruit showed highest ethylene climacteric peak rates during both 150 days (2.11 µmol kg^{-1} h^{-1}) and 200 days (1.51 µmol kg^{-1} h^{-1}) of CA storage when compared to all other ethylene antagonist treatments (Table 8.1).

The onset of ethylene climacteric peak was significantly delayed in the Gold Rush pear fruit fumigated with 1-MCP during both 150 and 200 days of CA storage, but no significant difference was recorded among other treatments (Table 8.1).

8.3.1.2 Respiration (CO_2 production)

Similar to the rates of ethylene climacteric peak, the respiration climacteric peak rates were also significantly lowered by the ethylene antagonist (BC, NC and 1-MCP) fumigation treatments following both the CA storage periods of 150 and 200 days (Table 8.1). The highest rates of respiration climacteric peak were exhibited by control fruit for both 150 days (0.84 mmol kg^{-1} h^{-1}) and 200 days (0.71 mmol kg^{-1} h^{-1}) of CA storage.

There was no significant effect of the ethylene antagonist treatments on the onset of respiratory climacteric peaks following both 150 and 200 days of CA storage.

Table 8.1 Effect of the ethylene antagonists on the climacteric peak onset (days) and peak rate of ethylene (µmol $kg^{-1}h^{-1}$) and respiration (mmol $kg^{-1}h^{-1}$) of Gold Rush pear fruit stored in CA storage.

	Storage period (days)			
Treatment	150		200	
	C_2H_4 climacteric peak onset	C_2H_4 climacteric peak rate	C_2H_4 climacteric peak onset	C_2H_4 climacteric peak rate
Control	3.00±0.35a	2.11±0.18c	4.00±0.00a	1.51±0.13c
BC	4.25±0.89a	1.52±0.11b	6.50±0.90a	1.09±0.10b
NC	5.50±0.75a	1.42±0.03b	4.50±0.25a	1.25±0.06b
1-MCP	9.25±0.65b	0.02±0.02a	9.00±0.87b	0.02±0.01a
LSD ($P \leq 0.05$)	2.69	0.41	2.47	0.23
	Respiration climacteric peak onset	Respiration climacteric peak rate	Respiration climacteric peak onset	Respiration climacteric peak rate
Control	4.75±0.22	0.84±0.03c	5.00±0.35	0.71±0.03c
BC	6.75±0.54	0.67±0.01b	5.50±0.43	0.55±0.04b
NC	6.25±0.41	0.70±0.05b	5.75±0.96	0.62±0.04b
1-MCP	6.25±0.41	0.48±0.04a	4.50±0.25	0.32±0.02a
LSD ($P \leq 0.05$)	ns	0.14	ns	0.09

ns = non-significant, n = 4 replicates (2 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns. Mean values followed by a similar letter are not significantly different within the columns. Mean values without letters within columns are non-significant. 150- and 200-days data were analysed separately.

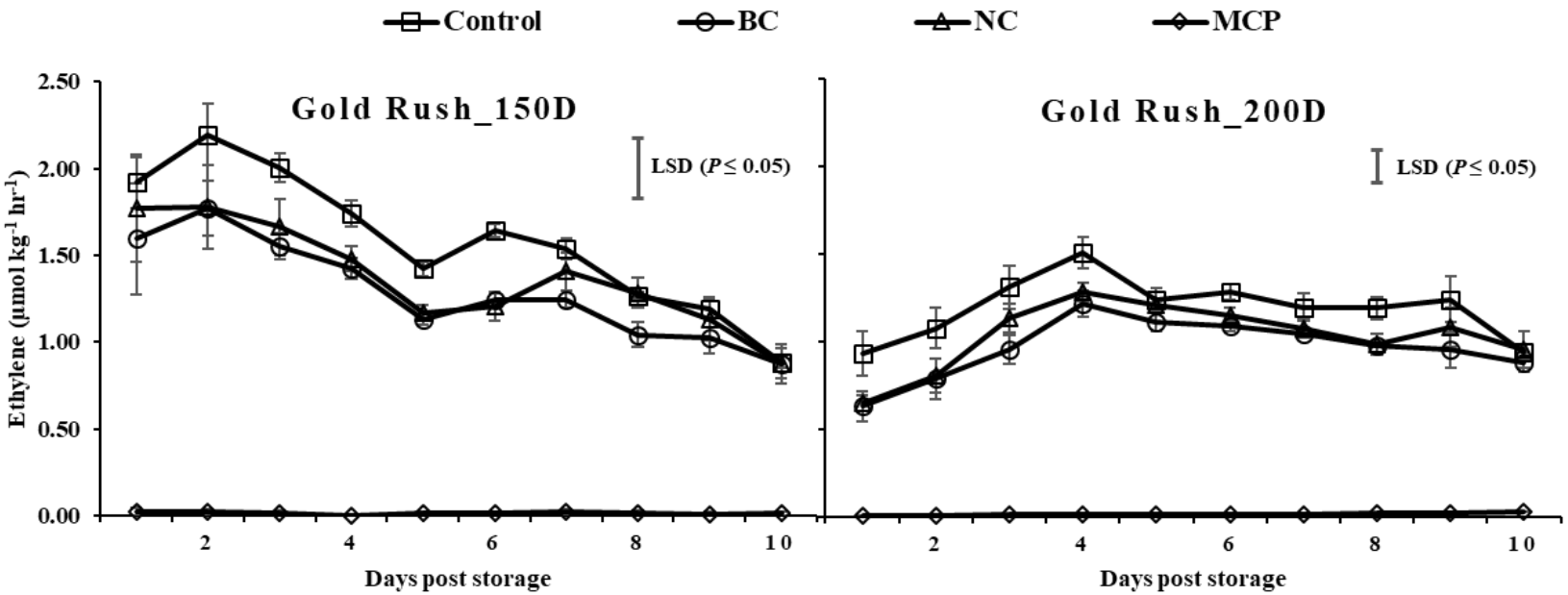


Figure 8.1 Changes in the rate of ethylene production due to ethylene antagonist fumigation treatment (T) during ripening days (D) in Gold Rush pear fruit stored for 150 and 200 days in CA storage. Vertical bars represent SE of mean values and are not visible when values are smaller than the symbol. n=4 replicates (2 fruit per replication). LSD ($P \leq 0.05$) T=0.11, D=0.17, TXD=0.35 for 150 days and T=0.06, D=0.10, TXD=0.19 for 200 days.

8.3.2 Fruit quality parameters

8.3.2.1 Physiological loss of weight (PLW)

The Gold Rush pear fruit fumigated with ethylene antagonists (BC, NC and 1-MCP) exhibited a significantly lower mean PLW value (1.52 %, 1.49 % and 1.11 %, respectively) in comparison to the untreated fruit (2.29 %) (Table 8.2). The PLW % was higher in the pear fruit stored for 200 days when compared to the fruit stored for 150 days in CA storage (Table 8.2). No significant interaction effect between the storage period and ethylene antagonist treatments on the PLW was recorded.

8.3.2.2 Fruit firmness

The mean fruit firmness of the Gold Rush pear fruits fumigated with BC, NC and 1-MCP was significantly higher (74.94 N, 74.75 N and 75.25 N, respectively) when compared to the control fruit (70.05 N) (Table 8.2). The fruit firmness was significantly lower in the fruit stored for 200 days (71.91 N) when compared to the fruit stored for 150 days (75.58 N) in the CA storage (Table 8.2). There was no significant interaction effect between the storage period and ethylene antagonist treatments on the fruit firmness.

8.3.2.3 Soluble solids concentration (SSC), titratable acidity (TA) and SSC: TA

The Gold Rush pear fruit fumigated with BC and NC exhibited significantly lower (11.61 % and 11.70 %, respectively) mean SSC values when compared to control fruits and fruits treated with 1-MCP (Table 8.3). The pear fruit stored for 200 days exhibited significantly higher (11.95 %) SSC values when compared to the fruit stored for 150 days (11.54 %) in CA storage. There was a significant interaction effect between CA storage duration and ethylene antagonist treatment on the SSC levels in Gold Rush pear fruit. The pear fruit fumigated with BC and stored for 150 days in CA storage exhibited significantly lowest (11.35 %) SSC values when compared to all other treatments (Table 8.2). There was no significant effect of ethylene antagonist treatments or storage durations on the mean TA and SSC: TA values.

Table 8.2 Effect of the ethylene antagonists on the physiological loss of weight (PLW) (%) and fruit firmness (N) of the Gold Rush pear fruit stored in CA storage.

	Storage period (days)		
Treatment	150	200	Mean (T)
	Physiological loss of weight (PLW)		
Control	2.15±0.31	2.43±0.21	2.29B
BC	1.34±0.19	1.71±0.08	1.52A
NC	1.32±0.40	1.67±0.20	1.49A
1-MCP	1.11±0.38	1.11±0.30	1.11A
Mean (D)	1.48	1.73	
LSD ($P \leq 0.05$)	T=0.63	D=ns	TXD=ns
	Fruit firmness		
Control	70.34±0.54	69.76±1.65	70.05A
BC	77.70±2.14	72.17±1.24	74.94B
NC	76.69±1.81	72.81±0.80	74.75B
1-MCP	77.61±0.83	72.90±1.19	75.25B
Mean (D)	75.58B	71.91A	
LSD ($P \leq 0.05$)	T=3.32	D=2.35	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (15 fruit (PLW) and 10 fruit (fruit firmness) per replication), mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). Mean values followed by a similar letter are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant.

Table 8.3 Effect of the ethylene antagonists on the changes in the soluble solids concentration (SSC) (%), titratable acidity (TA) (%) and SSC: TA of the juice of Gold Rush pear fruit stored in CA storage

	Storage period (days)		
Treatment	150	200	Mean (T)
	Soluble solids concentration (SSC)		
Control	11.63±0.02b	12.05±0.02d	11.84B
BC	11.35±0.02a	11.88±0.02c	11.61A
NC	11.58±0.02b	11.83±0.02c	11.70A
1-MCP	11.60±0.09b	12.05±0.02d	11.83B
Mean (D)	11.54A	11.95B	
LSD ($P \leq 0.05$)	T=0.10	D=0.07	TXD=0.14
	Titratable acidity (TA)		
Control	0.06±0.01	0.07±0.01	0.07
BC	0.07±0.01	0.09±0.01	0.08
NC	0.08±0.00	0.09±0.01	0.09
1-MCP	0.08±0.01	0.09±0.01	0.08
Mean (D)	0.07	0.08	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=ns
	SSC: TA		
Control	217.04±36.38	174.85±12.50	195.95
BC	176.31±30.25	132.96±7.53	154.63
NC	143.97±0.27	132.40±7.50	138.18
1-MCP	161.55±17.48	142.41±6.65	151.98
Mean (D)	174.71	145.66	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

8.3.2.4 Individual sugars

The Gold Rush pear fruit fumigated with 1-MCP exhibited significantly highest mean glucose and sorbitol levels (51.84 g kg^{-1} and 118.64 g kg^{-1}, respectively) and lowest mean sucrose levels (66.77 g kg^{-1}) when compared to control fruit or the fruit fumigated with BC or NC (Table 8.4). There was no significant difference among the fruits fumigated with BC or NC and control fruit for the levels of glucose, sucrose and sorbitol (Table 8.4). There was no significant effect of ethylene antagonist treatments or CA storage duration on levels of fructose in Gold Rush pear fruit.

The levels of sucrose were significantly higher in the pear fruit stored for 150 days, while sorbitol levels were higher in the fruit stored for 200 days (Table 8.4). The pear fruit fumigated with 1-MCP and stored for 200 days in the CA storage exhibited significantly highest glucose levels when compared to all other treatments (Table 8.4). There was no significant interaction effect between storage period and ethylene antagonist treatment on the levels of fructose, sucrose and sorbitol.

8.3.2.5 Individual organic acids

Considerable amounts of malic acid, succinic acid and fumaric acid, while no tartaric acid and citric acid were identified by RP-HPLC system in the Gold Rush pear fruit samples. There was no significant effect of ethylene antagonists or CA storage duration on levels of malic acid and fumaric acid. The levels of succinic acid were significantly lower (3.24 g kg^{-1}) in the pear fruit stored for 200 days in CA storage, but there was no significant effect of ethylene antagonistic treatments (Table 8.5).

Table 8.4 Effect of the ethylene antagonists on the levels of individual sugars (g kg^{-1}) in the pulp of the Gold Rush pear fruit stored in CA storage

Storage period (days)			
Treatment	150	200	Mean (T)
Glucose			
Control	45.50±1.94b	49.95±1.82bc	47.73B
BC	45.48±0.59b	45.28±1.07b	45.38AB
NC	47.96±0.72b	39.61±1.46a	43.79A
1-MCP	50.48±1.93bc	53.20±0.96c	51.84C
Mean (D)	47.35	47.01	
LSD ($P \leq 0.05$)	T=3.42	D=ns	TXD=4.83
Fructose			
Control	305.29±3.97	308.73±1.64	307.01
BC	313.90±2.10	302.05±4.86	307.98
NC	301.55±4.93	308.71±4.30	305.13
1-MCP	312.42±12.31	316.29±7.33	314.35
Mean (D)	308.29	308.95	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=ns
Sucrose			
Control	104.16±6.67	87.07±6.90	95.61B
BC	112.23±5.75	87.56±2.44	99.89B
NC	99.47±1.76	86.64±2.41	93.06B
1-MCP	80.44±7.24	53.11±2.03	66.77A
Mean (D)	99.07B	78.59A	
LSD ($P \leq 0.05$)	T=12.04	D=8.51	TXD=ns
Sorbitol			
Control	99.01±2.57	111.30±2.09	105.15A
BC	98.44±1.17	109.37±2.12	103.90A
NC	95.71±2.56	114.40±1.17	105.05A
1-MCP	117.63±5.28	119.64±5.04	118.64B
Mean (D)	102.70A	113.68B	
LSD ($P \leq 0.05$)	T=7.79	D=5.51	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

Table 8.5 Effect of the ethylene antagonists on the levels of individual organic acids (g kg^{-1}) in the pulp of the Gold Rush pear fruit stored in CA storage

	Storage period (days)		
Treatment	150	200	Mean (T)
	Malic acid		
Control	1.25±0.13	0.98±0.06	1.11
BC	1.22±0.08	1.25±0.14	1.23
NC	1.31±0.30	1.35±0.06	1.33
1-MCP	1.36±0.22	1.22±0.38	1.29
Mean (D)	1.28	1.20	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=ns
	Succinic acid		
Control	4.30±0.20	2.66±0.60	3.48
BC	4.52±0.26	3.55±0.32	4.03
NC	4.13±0.52	2.83±0.33	3.48
1-MCP	3.33±0.55	3.93±0.26	3.63
Mean (D)	4.07B	3.24A	
LSD ($P \leq 0.05$)	T=ns	D=0.73	TXD=ns
	Fumaric acid		
Control	0.29±0.04	0.28±0.05	0.29
BC	0.19±0.00	0.21±0.02	0.20
NC	0.24±0.01	0.26±0.03	0.25
1-MCP	0.20±0.01	0.23±0.04	0.22
Mean (D)	0.23	0.25	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

8.3.2.6 Total phenols, ascorbic acid and total antioxidant capacity

The ethylene antagonist treatments did not show any significant effect on the levels of total phenols, ascorbic acid and total antioxidant capacity. The mean levels of ascorbic acid and total antioxidant capacity were significantly lower (4.39 g kg^{-1} and 3.74 µM kg^{-1} Trolox, respectively) in the Gold Rush pear fruit stored for 200 days when compared to the 150 days stored fruit in the CA storage (Table 8.6). There was no significant interaction effect between storage period and ethylene antagonist treatment on the levels of total phenols, ascorbic acid and total antioxidant capacity.

Table 8.6 Effect of the ethylene antagonists on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of the Gold Rush pear fruit stored in CA storage

Storage period (days)			
Treatment	150	200	Mean (T)
Total phenols (g GAE kg^{-1})			
Control	11.41±0.73	12.16±1.23	11.79
BC	12.16±0.82	12.82±2.58	12.49
NC	11.04±0.49	10.20±0.87	10.62
1-MCP	10.29±0.82	10.29±0.50	10.29
Mean (D)	11.23	11.37	
LSD ($P \leq 0.05$)	T=ns	D=ns	TXD=ns
Ascorbic acid (g kg^{-1})			
Control	5.26±0.18	4.56±0.44	4.91
BC	5.12±0.29	4.88±0.34	5.00
NC	4.81±0.24	4.14±0.05	4.48
1-MCP	5.47±0.19	3.97±0.25	4.72
Mean (D)	5.17B	4.39A	
LSD ($P \leq 0.05$)	T=ns	D=0.44	TXD=ns
Total antioxidant capacity (µM kg^{-1} Trolox)			
Control	5.31±0.46	3.95±0.19	4.63
BC	5.72±0.33	3.76±0.22	4.74
NC	4.68±0.22	3.60±0.13	4.14
1-MCP	5.50±0.28	3.65±0.10	4.57
Mean (D)	5.31B	3.74A	
LSD ($P \leq 0.05$)	T=ns	D=0.45	TXD=ns

ns = non-significant, T = treatments, D = storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

8.4 Discussion

The effects of fumigation with ethylene antagonists (BC, NC and 1-MCP) on the postharvest physiology and quality parameters of the Gold Rush pear fruit stored in CA storage were investigated in the present experiment. The treatment with BC, NC and 1-MCP has effectively reduced ethylene and respiratory climacteric peak rates in the Gold Rush pear fruits during CA storage when compared to control fruit. These results suggest all the ethylene antagonist compounds were effective in blocking the ethylene receptors, counteract the effects of ethylene and in retarding the autocatalytic ethylene production in the fruit (Sisler, 2006). The antagonistic effect is attributed to

the capacity of the compounds to block the ethylene receptor sites and hinder the expression of ethylene response genes in the fruits (Sisler et al., 2003; Apelbaum et al., 2008; Musa, 2016). Various ripening-associated physical, physiological and biochemical changes in the fleshy fruit are directly or indirectly controlled by ethylene hormone (Imaseki, 1991; Anwar et al., 2018).

The Gold Rush pear fruit fumigated with ethylene antagonists (BC, NC and 1-MCP) showed significantly lowest PLW in comparison with control fruit. The weight loss in fruit during storage is mainly due to loss of water from the fruit as a result of continuous postharvest physiological processes such as respiration and transpiration (Becker and Fricke, 1996). The higher rates of respiration during storage result in higher transpiration of water from the fruit surface and thus increase in PLW (Dhillon and Mahajan, 2011). The reduction in ethylene and respiratory rates could be related to a decrease in the levels of PLW in the fruit (Martínez-Romero et al., 2007).

The fruit firmness is closely related to the degree of ripeness, internal quality and probable shelf-life in pears (Zhang et al., 2018). The Gold Rush pear fruit fumigated with BC, NC and 1-MCP maintained significantly higher mean fruit firmness when compared to control fruit during CA storage. The ideal fruit firmness of pear fruit range from 50 to 58 N. The hydrolysis of cell wall constituents and loss of cell turgidity are the main factors responsible for the loss of fruit firmness during storage (Van Buren, 1979; Giovannoni, 2008). The plant hormone ethylene is involved in the activation of cell wall hydrolysing enzymes in the fruit (Giovannoni, 2008). The maintenance of higher fruit firmness in ethylene antagonist treated fruit can be associated to a reduction in rates of ethylene and respiration as well as a decrease in PLW (Harker et al., 1997; Giovannoni, 2008). The fruit firmness in the pear fruit decreased with the increase in the storage period. This is due to continuous physiological activities occurring in the fruit during extended storage life. A similar reduction in fruit firmness in pear fruit was recorded by Argenta et al. (2016).

The levels of SSC were maintained significantly lowest in pear fruit treated with BC and NC. Fruit ripening process involves the breakdown of acids into sugars to meet the demand for primary substrates in the respiration process. The lower levels of SSC in the fruit can be related to lowered rates of respiration (Tucker, 2012). The rise in SSC with extension in storage duration is due to the continuous process of conversion

of acids into sugars during storage (Tucker, 2012). The levels of TA were not significantly affected by the ethylene antagonist treatment. Similar observations were recorded by Calvo and Sozzi (2004) in Red Clapp's pear, Larrigaudiere et al. (2004) in Blanquilla pears and Trinchero et al. (2004) in Bartlett pears.

The levels of glucose and sorbitol were significantly highest in fruit treated with 1-MCP, but the sucrose levels were significantly lowest in comparison with other treatments. These variations can be attributed to the interconversions of sugars during the ripening process (Kader, 1980). Overall, the levels of sugars in the 1-MCP treated fruit are higher than all other treatments. Similar observations of sugar levels in pear fruit were reported by Mahajan et al. (2010b). The levels of malic acid and succinic acid reduced with the extension of the storage period. The reduction in organic acid levels is due to the breakdown of acids to convert into simple sugars. These simple sugars are consumed as respiratory substrates during fruit ripening processes, which continue during storage (Giovannoni, 2008).

The levels of ascorbic acid and total antioxidant capacity in the Gold Rush pear fruit declined with the extension of the storage period. The fruit ripening process involves several oxidative reactions with the production of reactive oxidative species (ROS) (Masia, 1998). The bioactive compounds such as ascorbic acid with anti-oxidant capacity is mainly involved in degrading the ROS formed and hence get used up with the progression of ripening process (Stenite et al., 2004; Valero et al., 2016).

8.5 Conclusions

The fumigation treatment with ethylene antagonists (BC, NC and 1-MCP) effectively reduced the rates of ethylene and respiration in the Gold Rush pear fruit following both 150 and 200 days of CA storage. The ethylene antagonist treatments maintained higher fruit firmness and lower PLW. The pear fruit fumigated with BC and NC exhibited lower levels of SSC, glucose and sorbitol when compared to control and 1-MCP treated fruit. The new ethylene antagonists possess the potential to be used as an alternative to 1-MCP treatment in maintaining fruit quality of Gold Rush during CA storage. The effects of the different concentrations of the new ethylene antagonists warrant further investigation to retard the levels of ethylene peaks as efficiently as 1-MCP.

CHAPTER 9

General discussion, conclusions, limitations and future prospects

9.1 Introduction

It is estimated that one-third of the total food produced globally for human consumption is either lost or wasted before it reaches the consumer. Amongst the total postharvest losses, it is estimated that nearly 44 % of losses are in fruits and vegetables (Lipinski et al., 2013). In Australia, the postharvest losses amongst fruit and vegetables range from 10 to 35 % and the total cost of agricultural food losses is estimated to be A$ 2.84 billion per annum (Lapidge 2015). Compared to grain crops, fruits and vegetables are highly susceptible to rapid quality deterioration. The increased rates of postharvest losses in the horticulture crops are mainly due to the higher rates of physiological activities which further lead to senescence and deterioration (Kader, 2004). The postharvest losses have a negative impact on the world economic, sociological and environmental factors and reducing these losses is considered as one of the sustainable methods to achieve global food security (Kitinoja et al., 2018). Blanke (2014) has identified that nearly 50 % of all postharvest losses in the horticulture crops is due to physical, physiological and biochemical changes associated with the fruit ripening process. Phytohormone ethylene plays a major role to promote these irreversible changes occurring during fruit ripening (Anwar et al., 2018). Appropriate postharvest handling, optimum storage conditions and effective ethylene regulation can significantly delay fruit ripening process and hence reduce postharvest losses as well as maintain optimum fruit quality during storage (Gross et al., 2016).

Cold storage and controlled atmospheric (CA) storage are widely being used to extend the storage life of fruits all around the world. The low temperatures and modified CO_2 and O_2 concentrations in the storage rooms significantly reduce the rate of fruit physiological activities and hence elongate the storage life while maintaining relatively good fruit quality (Keller et al., 2013; Gross et al., 2016). The response of fruit to these storage environments vary according to species, cultivar, pre- and postharvest practices and storage duration. Above all, the storage conditions can not directly regulate the ethylene action in the fruit and in the storage environment. Hence different interventions are used along with these storage conditions to manage external and internal ethylene production in fruit and its accumulation in storage rooms.

Application of ozone gas in the storage rooms oxidise the external ethylene present in the storage environment and minimise the effect of ethylene on the fruits (Skog and Chu, 2001). Similarly, recent innovation AiroFresh® working on photocatalytic oxidation (PCO) principle completely oxidise and degrade the ethylene in the storage environment (AiroFresh®, 2019). Cold storage and CA storage combined with ozone application or AiroFresh® will be useful to reduce the ethylene in storage rooms to a significant level and thus improve storage life as well as maintain fruit quality for a long time. But these treatments would not have any effect on the internal ethylene production and ethylene action due to the expression of genes, which are activated in response to ethylene exposure even at very low concentrations (Skog and Chu, 2001; Anwar et al., 2018). Hence the application of ethylene antagonists along with the combination of different storage conditions will assure effective control of internal ethylene production and its action in the fruits as well as degrade external ethylene to extend storage life and maintain the relatively ideal fruit quality.

Different methods have been tested by several researchers to antagonise the ethylene action in the fruits and vegetables. Compared to all the methods 1-methylcyclopropene (1-MCP) has been proven to be the most effective ethylene antagonist and stable cyclopropene in the gaseous state (Sisler et al., 2006). 1-MCP antagonises ethylene action at the cellular level by irreversibly blocking the ethylene receptor sites in the fruits and further prevent the expression of ethylene-responsive genes (Sisler, 2006). But 1-MCP boils at temperatures as low as 0°C and is an unstable liquid at room temperatures and vaporise rapidly (Sisler et al., 2006). It is available only as an expensive service and not affordable to many growers all around the world. There is a need to develop ethylene antagonist compounds as effective as 1-MCP in regulating the rates of ethylene production and respiration as well as maintain consumer-preferred fruit quality during storage. The new compounds should as well address the limitations of 1-MCP. The two new potential ethylene antagonist compounds namely 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) were developed by Singh et al. (2018). It has been proposed that these compounds antagonise the ethylene action in the fruits through the mode of action similar to 1-MCP (Pirrung et al., 2008; Musa, 2016).

In the present work, for the first time the effects of BC, NC and 1-MCP on the internal ethylene production and the fruit quality of Granny Smith apple, Cripps Pink apple

and Gold Rush pear fruits stored in cold or CA storage with or without ozone application or AiroFresh® installation were investigated. The experiments were designed according to the storage conditions being used by the fruit growers, so that the results are more relevant and applicable to the industry. It is hypothesised that these treatments will effectively reduce the rates of ethylene production and respiration, decrease PLW, maintain optimum fruit firmness, higher levels of bioactive compounds and other fruit quality parameters. The success of this project will provide the global horticulture industry with ethylene antagonists, which are more stable than and as effective as 1-MCP in antagonising the ethylene action at different storage environments and storage durations.

9.2 General discussions

All the ethylene antagonist compounds BC, NC and 1-MCP delayed the onset and reduced the rates of ethylene climacteric peak in Granny Smith apple, Cripps Pink apple and Gold Rush pear fruits stored under different storage environments and durations. This determines the capacity of the ethylene antagonist compounds to irreversibly block the ethylene receptors, impede the expression of ethylene-responsive genes and inhibit autocatalytic ethylene production in the fruits (Sisler et al., 2003; Apelbaum et al., 2008; Musa, 2016). The physical, physiological and bioactive quality parameters of the fruit during the storage are directly or indirectly affected by the ethylene and respiration levels (Anwar et al., 2018). But the extent of the influence of ethylene and ethylene antagonists on these fruit quality parameters may be different depending upon factors such as species, cultivar, cultivation practices, maturity at harvest, postharvest handling as well as storage environment and duration (Abeles et al., 1992; Watkins, 2006). The effect of ethylene antagonist treatments on the rates of respiration and ethylene production and also on other fruit quality parameters in the different storage conditions are concisely discussed below.

9.2.1 Different formulations of new ethylene antagonist compounds

The first objective of the study was to elucidate the effect of different formulations of new potential ethylene antagonists on ethylene production rate and fruit quality of Cripps Pink apple stored in the cold storage. The widely used ethylene antagonist 1-MCP is an unstable liquid even at temperatures as low as 0 °C and this makes it not feasible to prepare for dip treatments (Sisler et al., 2006). It was hypothesised that all

the ethylene antagonist formulations would effectively retard the rates of respiration and ethylene production as well as maintain consumer-preferable fruit quality following 100 and 150 days of cold storage. The findings of this experiment would enable the opportunity to treat the fruit with ethylene antagonists by fruit dip and fumigation methods in order to extend storage life and maintain optimum fruit quality during storage. In agreement to the hypothesis, when compared to control and ethanol treatments, the Cripps Pink fruit treated with BC, NC and 1-MCP exhibited reduced rates of ethylene production (Chapter 4, Figure 4.1), lowered PLW and maintained higher fruit firmness, TA and total phenol levels during both 100 and 150 days of cold storage. The observations determine that the ethylene antagonist compounds studied were effective in impeding the ethylene action (Sisler, 2006). The fumigation treatments with BC and NC showed better results than respective dip treatments in maintaining the fruit parameters studied. This difference in performance could be due to an insufficient concentration of chemical for dip treatment. Similarly, Argenta et al. (2007) also reported that the ethylene antagonistic effect of dip treatment with 1-MCP was equivalent to that of 1-MCP fumigation in the 'Golden Delicious' apple fruit only when the solution was made with concentration 700 times to that of fumigation. The efficiency of 1-MCP fumigation treatment to retard the rates of ethylene production and respiration in Cripps Pink fruit was comparatively higher when compared to BC and NC fumigation. In the present experiment, very low concentrations of BC (0.09 ppm) and NC (0.14 ppm) were applied as fumigation treatments when compared to 1-MCP (1.00 ppm). Hence studying the efficacy of different concentrations of BC and NC in reducing the rate of respiration and ethylene production warrants further investigation.

9.2.2 Combined effects of ethylene antagonists and ozone on the cold-stored Granny Smith and Cripps Pink apple fruits

The second objective of the investigation was to examine the combined effects of ozone and ethylene antagonist treatments in regulating the ethylene action and maintaining the postharvest fruit quality in Cripps Pink and Granny Smith apples stored in cold storage. A comparative study conducted between cold storage environments with ozone and without ozone. Ozone gas is a powerful oxidising agent well known for its biocidal properties (Tzortzakis and Chrysargyris, 2017). Skog and Chu (2001) reported that ozone possesses the capacity to oxidise ethylene present in

the storage environment but exhibited no significant effect on the levels of internal ethylene production in the fruit. Liew and Prange (1994) reported that ozone application is more effective with increased residual ozone concentrations when applied in low-temperature storage rooms. Similarly, Palou et al. (2001, 2002) also found a synergistic effect of ozone treatment and cold temperatures in retarding postharvest physiological changes associated with ethylene in citrus and peach fruits. However, there are no reports on the combined effects of ozone application and ethylene antagonist treatments on Cripps Pink and Granny Smith apple fruits stored in cold storage. It was hypothesised that combination of ethylene antagonist treatments and ozone gas in the cold storage would perform synergistically to retard rates of ethylene production and respiration as well as maintain optimum postharvest fruit quality during both 90 and 120 days of storage. The ethylene antagonist treatments lowered the rates of ethylene production and respiration, delayed climacteric peak onset (Chapter 5, Figure 5.1), reduced PLW and maintained higher fruit firmness, total phenols and total antioxidant capacity when compared to control fruit during both 90 and 120 days of storage in both Granny Smith and Cripps Pink apples. The observations represent that all the ethylene antagonist treatments effectively impeded the ethylene action in the Cripps Pink and Granny Smith fruits (Sisler, 2006). Cripps Pink fruit fumigated with BC and NC exhibited significantly lowest ethylene and respiration climacteric peak values when compared to 1-MCP treatment and control fruit (Table 5.1). In the case of the Granny Smith apple fruit, the fruit fumigated with BC and NC exhibited lower respiratory and ethylene peak values when compared to control, but the fruit treated with 1-MCP showed lowest values (Table 5.2). These observations reconfirm that the effects of ethylene antagonists on the postharvest fruit physiological parameters vary with different cultivars (Watkins, 2006). The ethylene peak rates were high in the apple fruit stored in the cold storage with ozone when compared to the ones stored in the cold storage without ozone application. Similar observations were recorded by Liew and Prange (1994) and explained that it is due to the stress-induced rise in ethylene production at high ozone concentrations. Reduced PLW and increased individual sugar levels were recorded in the apple fruit stored in the ozonated cold storage. There was no significant interaction effect between ethylene antagonist treatment and ozone application in the cold storage on the rates of ethylene production and respiration as well as on other fruit quality parameters studied. The novel ethylene antagonists (NC and BC) possess the capacity to become an alternative

to 1-MCP treatment. Studying effects of different concentrations of new ethylene antagonists on the fruit physiological and quality parameters in various cultivars would allow standardising respective best effective concentrations.

9.2.3 Combined effects of ethylene antagonists and AiroFresh® technology on the Cripps Pink fruit stored in controlled atmosphere storage

Elucidating the combined effects of ethylene antagonist treatments and AiroFresh® technology in the Cripps Pink apple fruit stored in the CA storage was the third objective of the research. AiroFresh® is a relatively new technology which utilises advanced oxidation processes (AOP) and photocatalytic oxidation (PCO) technique to degrade volatile organic compounds like ethylene in the closed storage environment. The technology also claims to induce conditions suitable for enhancing the efficiency of ethylene antagonists (AiroFresh®, 2019). There are no reports on the combined effects of ethylene antagonist treatments and AiroFresh® technology on the postharvest physiology and fruit quality of Cripps Pink apple fruit stored in CA storage. It was hypothesised that the combination of ethylene antagonist treatments and AiroFresh® technology in the CA storage will synergistically reduce ethylene production and respiration rates as well as maintain optimum fruit quality following different storage durations. The ethylene antagonistic treatments delayed the onset and also reduced the rates of the climacteric peak of respiration and ethylene in Cripps Pink apple fruit stored in CA storage (Figure 6.1; Figure 7.1 C-D). The treatments also lowered PLW and maintained higher fruit firmness, SSC, individual sugars, total phenols and total antioxidant capacity when compared to control fruit during both 90 and 120 days of CA storage in the Cripps Pink apples (Chapter 6). Following 90 and 120 days of the storage, the fumigation treatment with NC exhibited results on par with 1-MCP treatment in retarding rates of ethylene production and respiration, reducing PLW and maintaining higher fruit firmness, total phenol and total antioxidant capacity (Chapter 6). Similarly, the fruit treated with BC, NC and 1-MCP exhibited delayed onset and retarded rates of ethylene climacteric peak as well as higher fruit firmness, SSC, TA, malic acid and total phenols when compared to control fruit following 60 and 150 days of storage in CA with AiroFresh® (Chapter 7). The fumigation treatment with BC and NC showed results on par with 1-MCP treatment in reducing rates of ethylene production and maintaining the fruit quality parameters

studied (Chapter 7). These observations could be related to the effective ethylene antagonist activity of the compounds used (Sisler, 2006).

Following 90 days of storage, the apple fruit stored in the CA storage installed with AiroFresh® exhibited reduced rates of ethylene and respiration, higher fruit firmness and total antioxidant capacity levels and lower PLW when compared to fruit stored in CA storage without AiroFresh®. But the results were contrary following 120 days of storage. The performance of the instruments working on photocatalytic oxidation principle depends upon the availability of O_2 gas and effective oxidisation of ethylene gas occurs only when the concentrations of O_2 are high enough (Pathak et al., 2017). The levels of O_2 are maintained low in the CA storage (Gross et al., 2016) and the reduction in the efficiency of AiroFresh® equipped in the CA storage at 120 days of storage could be due to availability of insufficient amounts of O_2. There was no significant interaction effect between ethylene antagonist treatments and type of CA storage on the respiration and ethylene rates as well as other fruit quality parameters studied. The new ethylene antagonist NC and BC possesses the ability to be used as an alternative to 1-MCP to counteract ethylene action in the Cripps Pink fruit during CA storage.

9.2.4 Postharvest fumigation of ethylene antagonists in the Granny Smith apple fruit stored in controlled atmospheric storage

The fourth objective of the study was to investigate the effects of ethylene antagonist fumigation treatments on the rates of ethylene production and in maintaining consumer preferable fruit quality in the Granny Smith apple stored in CA storage. It was hypothesised that all the ethylene antagonist treatments would retard the rates of ethylene production and respiration as well as maintain optimum postharvest fruit quality following both 90 and 120 days of storage. The fumigation treatment with BC, NC and 1-MCP reduced the rate and delayed the onset of respiratory and ethylene climacteric peaks in Granny Smith apple fruit following 90 and 120 days of CA storage in comparison to the control fruit (Figure 7.1 and Table 7.1). These observations could be correlated with the efficient activity of ethylene antagonist treatments (Sisler, 2006). The fruit treated with ethylene antagonists retained higher fruit firmness, exhibited lower PLW and TA and higher levels of SSC, individual sugars and acids, total phenols, ascorbic acid as well as total antioxidant capacity when compared to

control fruit (Chapter 7). The fumigation treatments of NC and BC effectively lowered the rates of ethylene production and respiration as well as maintained optimum fruit quality when compared to control fruit. But the fruit treated with 1-MCP performed best over all other treatments. Hence there is a need for further investigation with different concentrations of BC and NC to achieve the best performance and to be used as an alternative to 1-MCP in Granny Smith apple fruit in CA storage.

9.2.5 Ethylene antagonist treatment in the Gold Rush pear fruit stored in controlled atmospheric storage

Investigating the effects of ethylene antagonist treatments on regulating the ethylene action and maintaining the consumer preferable fruit quality of Gold Rush pear stored in CA storage is the fifth objective of the study. The experiment was conducted with the hypothesis that all the ethylene antagonists will effectively retard rates of ethylene production and respiration as well as retain the consumer preferable fruit quality in the Gold Rush pear, during both 150 and 200 days of CA storage. The fumigation treatment with the ethylene antagonists significantly reduced rates of ethylene and respiratory climacteric peaks (Figure 8.1 and Table 8.1) as well as maintained higher fruit firmness and lower PLW compared to control following both 150 and 200 days of CA storage (Chapter 8). These results could be related with the effective activity of all the ethylene antagonist compounds tested in impeding the ethylene action in the Gold Rush pear fruit (Sisler, 2006). The values of the SSC, glucose and sorbitol were lower in the fruit fumigated with BC and NC when compared to 1-MCP treatment and control fruit. The 1-MCP fumigation retarded ethylene and respiration rates more effectively when compared to BC and NC. The new ethylene antagonists were equally effective in maintaining the postharvest fruit quality parameters studied. The BC and NC have the potential to be used as ethylene antagonist alternative to 1-MCP. There is a need for further investigation using different concentrations of BC and NC on the Gold Rush pears to achieve the best performance and effectively retard the ethylene and respiration rates similar to that of 1-MCP stored at CA storage.

9.3 Conclusions and recommendations

- The fumigation with 1 µM BC (0.09 µL L^{-1}) or 1 µM NC (0.14 µL L^{-1}) effectively reduced the rates of ethylene and respiration peaks in Granny Smith apple, Cripps Pink apple and Gold Rush pear fruits stored in the different

storage environments and all the durations studied. But freshly prepared 1-MCP (1 µL L^{-1}) fumigation was comparatively more effective than BC and NC in retarding the ethylene and respiration rates.

- Fumigation treatment with 1 µM BC or 1 µM NC was better than aqueous dip treatment of 2 µM BC + 5% ethanol or 2 µM NC + 5% ethanol in antagonising ethylene activity in the Cripps Pink apple fruit.
- The application of ozone gas in the cold storage aided in maintaining fruit quality for the parameters studied but induced a rise in rates of ethylene production in the Cripps Pink and Granny Smith apple fruit. There was no significant interaction effect between ethylene antagonist treatment and ozone application in the cold storage.
- When compared to conventional CA storage, the CA storage with AiroFresh® technology exhibited better performance in retarding the rates of ethylene production and respiration as well as in maintaining the postharvest fruit quality following 90 days but not for 120 days of storage. There was no significant interaction effect between ethylene antagonist treatments and CA storage environments on the parameters investigated.

9.4 Limitations

- The ozone gas in the commercial storage was generated using a corona discharge method and the concentration of ozone gas produced varied from 0.03 to 0.3 µL L^{-1}. The differences in the concentrations of the ozone gas produced might be due to variations in the oxygen concentration in the cold storage.
- According to chemical nature, BC and NC would be safe for human consumption. The initial studies on toxicity are promising with NC being non-toxic to VERO and KB cancer cell lines (>100µM). But the official process to pass the 'certification for human consumption' is still in progress. Hence, no sensory evaluation could be conducted in this investigation.

9.5 Future prospects

- ✓ The efficacy of BC and NC in antagonising the ethylene action in the apple and pear fruits may be investigated in other commercial cultivars.

- ✓ The different concentrations of BC and NC fumigation can be tested in various fruit cultivars in order to achieve their best performance in retarding the rates of ethylene production and respiration similar to that of 1-MCP.
- ✓ The dip formulations with higher concentrations of BC and NC may be tested to standardise the best concentrations to treat the fruit by fruit dip to extend storage life and maintain optimum fruit quality during storage.
- ✓ The effect of the new ethylene antagonists on up or down-regulation of ethylene-responsive genes involved in fruit ripening is yet to be investigated.

Literature cited

Abdalghani, S.M.A., (2017). Regulation of vase life of waxflowers (*Chamelaucium* Desf.) with new ethylene antagonists. *Doctoral Dissertation*, Curtin University, WA.

Abdalghani, S.M.A., Singh, Z., Seaton, K., and Payne, A. (2018). (S)- (-)-limonene fumigation protects waxflowers (*Chamelaucium* spp.) from detrimental effects of ethylene on abscission of flowers/buds. *Australian Journal of Crop Science, 12*(12), 1875-1881.

Abdi, N., Holford, P., McGlasson, W.B., and Mizrahi, Y. (1997). Ripening behaviour and responses to propylene in four cultivars of Japanese type plums. *Postharvest Biology and Technology, 12*(1), 21-34.

Abeles, F.B. (1973) Ethylene in plant biology, 1st Edn, Academic Press Inc., NY.

Abeles, F.B., Morgan, P.W., and Saltveit, M.E. (1992) Ethylene in plant biology, 2nd Edn, Academic Press Inc., CA.

ABS. (2018). Australian Bureau of Statistics (ABS). Agricultural Commodities, Australia and state/territory. http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/7121.02016-17?OpenDocument. Retrieved 1st September 2018.

Ackermann, J., Fischer, M., and Amado, R. (1992). Changes in sugars, acids, and amino acids during ripening and storage of apples (cv. Glockenapfel). *Journal of Agricultural and Food Chemistry*, *40*(7), 1131-1134.

Adams, D.O., and Yang, S.F. (1977). Methionine metabolism in apple tissue: implication of S-adenosylmethionine as an intermediate in the conversion of methionine to ethylene. *Plant Physiology, 60*(6), 892-896.

Agar, I.T., Biasi, W.V., and Mitcham, E.J. (1999). Exogenous ethylene accelerates ripening responses in Bartlett pears regardless of maturity or growing region. *Postharvest Biology and Technology*, *17*(2), 67-78.

AgriFutures. (2019). AgriFutures Australia. https://www.agrifutures.com.au/farm-diversity/. Retrieved 5th December 2019.

Aguayo, E., Escalona, V.H., and Artés, F. (2006). Effect of cyclic exposure to ozone gas on physicochemical, sensorial and microbial quality of whole and sliced tomatoes. *Postharvest Biology and Technology*, *39*(2), 169-177.

AiroFresh® (2019). AiroFresh Intl® - Fresh Innovation Technology. http://airofreshintl.com/. Retrieved from 19th February 2019.

Ali, A., Ong, M.K., and Forney, C.F. (2014). Effect of ozone pre-conditioning on quality and antioxidant capacity of papaya fruit during ambient storage. *Food Chemistry*, *142*, 19-26.

Anwar, R., Matoo, A.K., and Handa, A.K. (2018). Ripening and senescence of fleshy fruits. In: *Postharvest Biology and Nanotechnology* (eds. Paliyath, G., Subramanian, J., Lim, L-T., Subramanian, K.S., Handa, A. and Matoo, A.) John Wiley and Sons Inc., pp. 15-53.

APAL. (2019). Apple and Pear Australia Limited (APAL). http://apal.org.au/industry-info/apple-and-pear-varieties/. Retrieved 20th February 2019.

Apelbaum, A., Sisler, E. C., Feng, X., and Goren, R. (2008). Assessment of the potency of 1-substituted cyclopropenes to counteract ethylene-induced processes in plants. *Plant Growth Regulation*, *55*(2), 101-113.

Argenta, L.C., Fan, X., and Mattheis, J.P. (2007). Responses of 'Golden Delicious' apples to 1-MCP applied in air or water. *HortScience*, *42*(7), 1651-1655.

Arteca, R.N. (2013). Plant growth substances: principles and applications. Springer Science and Business Media., pp. 73-81.

Artés-Hernández, F., Aguayo, E., Artés, F., and Tomás-Barberán, F.A. (2007). Enriched ozone atmosphere enhances bioactive phenolics in seedless table grapes after prolonged shelf life. *Journal of the Science of Food and Agriculture*, *87*(5), 824-831.

Artés-Hernández, F., Artés, F., and Tomás-Barberán, F.A. (2003). Quality and enhancement of bioactive phenolics in cv. Napoleon table grapes exposed to different postharvest gaseous treatments. *Journal of Agricultural and Food Chemistry*, *51*(18), 5290-5295.

Australian Dietary Guidelines. (2013). Eat for health – Australian Dietary Guidelines – Providing the scientific evidence for healthier Australian diets. https://www.eatforhealth.gov.au/sites/default/files/content/n55_australian_dietary_guidelines.pdf. Retrieved 18th May 2019.

Bagnato, N., Sedgley, M., Barrett, R., and Klieber, A. (2003). Effect of ethanol vacuum infiltration on the ripening of 'Cavendish bananas' cv. Williams. *Postharvest Biology and Technology*, *27*(3), 337-340.

Bai, J., Prange, R.K., and Toivonen, P.A. (2009). Pome fruits. In: *Modified and Controlled Atmospheres for the Storage, Transportation, and Packaging of Horticultural Commodities* (ed. Yahia, E.M.). CRC Press Boca Raton, pp. 267-286.

Bailey, P.S. (1978). Organic chemistry. In: *Ozonation in Organic Chemistry, vol. 1: Olefinic Compounds*, Academic Press, NY. pp. 272.

Baritelle, A.L., Hyde, G.M., Fellman, J.K., and Varith, J. (2001). Using 1-MCP to inhibit the influence of ripening on impact properties of pear and apple tissue. *Postharvest Biology and Technology*, *23*(2), 153-160.

Batur, S., and Çetinbaş, M. (2017). Pre-harvest application of retain (Aminoethoxyvinylglycine, AVG) influences pre-harvest drop and fruit quality of 'Williams' Pears. *Journal of Agricultural Sciences*, *23*(3), 344-356.

Baur, A.H., and Yang, S.F. (1972). Methionine metabolism in apple tissue in relation to ethylene biosynthesis. *Phytochemistry*, *11*(11), 3207-3214.

Bazarova, V.I. (1982). Use of ozone in storage of apples. *Food Science and Technology Abstracts* 14 (11): J1653.

Becker, B.R., and Fricke, B.A. (1996). Transpiration and respiration of fruits and vegetables, In: *New Developments in Refrigeration for Food Safety and Quality* (International Institute of Refrigeration, Paris, France, and American Society of Agricultural Engineers, St. Joseph, MI, 1996), pp. 110-121.

Bell, R.L., Quamme, H.A., Layne, R.E.C., and Skirvin, R.M. (1996). Pears. In *Fruit Breeding, Volume I: Tree and Tropical Fr*uits (eds. Janick, J. and Moore, J.N.), 441-514.

Beltran, F.J. (2003). Introduction. In: *Ozone Reaction Kinetics for Water and Wastewater Systems*, CRC Press, NY, pp. 1-4.

Bennett, A.B. and Della-Penna, D. (1987) Polygalacturonase gene expression in ripening tomato fruit. In: *Tomato Biotechnology* (eds. Nevins, D. and Jones, R.). Alan R. Liss Inc., NY, pp. 299–308.

Biale, J.B. and Young, R.E. (1981) Respiration and ripening in fruits – retrospect and prospect. In: *Recent Advances in the Biochemistry of Fruits and Vegetables* (eds. Friend, J. and Rhodes, M.J.C.). Academic Press, London, UK, pp. 1–39.

Billups, W.E., and Chow, W.Y. (1973). Naphtho [b] cyclopropene. *Journal of the American Chemical Society*, *95*(12), 4099-4100.

Blakey, R.J., Tesfay, S.Z., Mathaba, N., Bertling, I. and Bower, J.P. (2012). Some initial changes in 'Hass' avocado (*Persea americana* Mill.) physiology due to ethephon. *International Journal of Postharvest Technology and Innovation*, *2*(4), 334–344.

Blanke, M.M. (2014). Reducing ethylene levels along the food supply chain: a key to reducing food waste? *Journal of the Science of Food and Agriculture*, 94(12), 2357-2361.

Blankenship, S.M., and Dole, J.M. (2003). 1-Methylcyclopropene: A review. *Postharvest Biology and Technology*, *28*(1), 1-25.

Blattný C. (2003). Pears. In: B. Caballero, L.C. Trugo, P.M. Finglas (Eds.), *Encyclopedia of Food Sciences and Nutrition* (eds. Caballero, B., Trugo, L.C. and Finglas, P.M.)., Academic Press, London (2003), UK, pp. 4428-4433.

Bocci, V. (2011). Physical-chemical properties of ozone – natural production of ozone: the toxicology of ozone. *In*: Ozone: A New Medical Drug. Dordrecht: Springer. pp. 1-4.

Bondonno, N. P., Bondonno, C. P., Blekkenhorst, L. C., Considine, M. J., Maghzal, G., Stocker, R., Woodman, R. J., Ward, N. W., Hodgson, J. M., and Croft, K. D. (2018). Flavonoid-Rich Apple Improves Endothelial Function in Individuals at Risk for Cardiovascular Disease: A Randomized Controlled Clinical Trial. *Molecular Nutrition and Food Research*, *62*(3), 1700674.

Brand-Williams, W., Cuvelier, M.E., and Berset, C.L.W.T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*(1), 25-30.

Bregoli, A.M., Scaramagli, S., Costa, G., Sabatini, E., Ziosi, V., Biondi, S., and Torrigiani, P. (2002). Peach (*Prunus persica*) fruit ripening: aminoethoxyvinylglycine (AVG) and exogenous polyamines affect ethylene emission and flesh firmness. *Physiologia Plantarum*, *114*(3), 472-481.

Brodowska, A.J., Nowak, A., and Śmigielski, K. (2018). Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. *Critical Reviews in Food Science and Nutrition*, *58*(13), 2176-2201.

Brummell, D.A., and Harpster, M.H. (2001). Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology*, *47*(1-2), 311-339.

Burg, S.P., and Burg, E.A. (1962). Role of ethylene in fruit ripening. *Plant Physiology*, 37(2), 179.

Burg, S.P., and Burg, E.A. (1967). Molecular requirements for the biological activity of ethylene. *Plant Physiology*, *42*(1), 144-152.

Burg, S.P., and Thimann, K.V. (1959) The physiology of ethylene formation in apples. *Proceedings of the National Academy of Sciences*, *45*(3), 335-344.

Calvo, G., and Sozzi, G. O. (2004). Improvement of postharvest storage quality of 'Red Clapp's' pears by treatment with 1-methylcyclopropene at low temperature. *The Journal of Horticultural Science and Biotechnology*, *79*(6), 930-934.

Carrington, C.S., Greve, L.C., and Labavitch, J.M. (1993). Cell wall metabolism in ripening fruit. VI. Effect of the antisense polygalacturonase gene on cell wall changes accompanying ripening in transgenic tomatoes). *Plant Physiology*, *103*(2), 429-434.

Chen, P.M., Richardson, D.G., and Mellenthin, W.M. (1982). Differences in biochemical composition between Beurre, d'Anjou and Bose pears during fruit development and storage. *Journal of the American Society for Horticultural Science, 107* (5), 807-812.

Choi, S.T., Tsouvaltzis, P., Lim, C.I., and Huber, D.J. (2008). Suppression of ripening and induction of asynchronous ripening in tomato and avocado fruits subjected to complete or partial exposure to aqueous solutions of 1-methylcyclopropene. *Postharvest Biology and Technology*, *48*(2), 206-214.

Concha-Meyer, A., Eifert, J.D., Williams, R.C., Marcy, J.E., and Welbaum, G.E. (2015). Shelf life determination of fresh blueberries (*Vaccinium corymbosum*) stored under controlled atmosphere and ozone. *International Journal of Food Science*. doi: 10.1155/2015/164143.

Cosgrove, D.J. (2001). Wall structure and wall loosening. A look backwards and forwards. *Plant Physiology*, *125*(1), 131-134.

Dal Cin, V., Rizzini, F.M., Botton, A., and Tonutti, P. (2006). The ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in apple and peach fruit. *Postharvest Biology and Technology*, *42*(2), 125-133.

Daun, H. (2005). Produce colour and appearance. In *Produce Degradation: Pathways and Prevention* (eds. Lamikanra, O., Imam, S., and Ukuku, D.). CRC Press LLC, Danvers, MA, pp. 191- 222.

Dauny, P. T., and Joyce, D. C. (2002). 1-MCP improves storability of'Queen Cox' and 'Bramley' apple fruit. *HortScience*, *37*(7), 1082-1085.

Davalian, D., Garratt, P.J., Koller, W., and Mansuri, M.M. (1980). Strained aromatic systems. Synthesis of cyclopropabenzocyclobutenes, cyclopropanaphthocylobutenes, and related compounds. *The Journal of Organic Chemistry*, *45*(21), 4183-4193.

de La, H.M.A.J., and des Roseaux, C. (1904). *L'Ozone et ses applications industrielles*. Vve. Ch. Dunod. pp. 49.

DeEll, J.R., Khanizadeh, S., Saad, F., and Ferree, D.C. (2001). Factors affecting apple fruit firmness-a review. *Journal-American Pomological Society*, *55*, 8-26.

DeEll, J.R., Murr, D.P., Wiley, L., and Mueller, R. (2005) Interactions of 1-mcp and low oxygen ca storage on apple quality. *Acta Horticulturae*, *682*, 941-948.

DeEll, J.R., Saad, F., and Khanizadeh, S. (1999). Factors influencing apple fruit firmness. *Compact Fruit Tree*, *32*(2), 56-58.

Defilippi, B.G., Dandekar, A.M., and Kader, A.A. (2004). Impact of suppression of ethylene action or biosynthesis on flavor metabolites in apple (*Malus domestica* Borkh) fruits. *Journal of Agricultural and Food Chemistry*, *52*(18), 5694-5701.

DeLong, J.M., Prange, R.K., and Harrison, P.A. (2004). The influence of 1-methylcyclopropene on ‘Cortland’ and ‘McIntosh’ apple quality following long-term storage. *HortScience*, *39*, 1062-1065.

Dhillon, W.S., and Mahajan, B.V.C. (2011). Ethylene and ethephon induced fruit ripening in pear. *Journal of Stored Products and Postharvest Research*, *2*(3), 45-51.

Doerflinger, F.C., Nock, J.F., Miller, W.B., and Watkins, C.B. (2019). Preharvest aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP) effects on ethylene and starch concentrations of 'Empire' and 'McIntosh' apples. *Scientia Horticulturae*, *244*, 134-140.

Domínguez, I., Lafuente, M.T., Hernández-Muñoz, P., and Gavara, R. (2016). Influence of modified atmosphere and ethylene levels on quality attributes of fresh tomatoes (*Lycopersicon esculentum* Mill.). *Food Chemistry*, *209*, 211-219.

Dondini, L., and Sansavini, S. (2012) European pear. In: *Fruit Breeding* (ed. Badenes, M.L., and Byrne, D.H.), Springer, NY. pp. 369-415.

E.P.A., 2002. Environmental Protection Agency (E.P.A.). Federal Register 67 (48) 796–48 800.

El-Shora, H.M., and Ap Rees, T. (1991). Intracellular location of $NADP^+$-linked malic enzyme in C_3 plants. *Planta*, 185(3), 362-367.

Escribano, S., Sugimoto, N., Macnish, A.J., Biasi, W.V., and Mitcham, E.J. (2017). Efficacy of liquid 1-methylcyclopropene to delay ripening of 'Bartlett' pears. *Postharvest Biology and Technology*, *126*, 57-66.

Espino-Díaz, M., Sepúlveda, D.R., González-Aguilar, G., and Olivas, G.I. (2016). Biochemistry of apple aroma: A review. *Food Technology and Biotechnology*, *54*(4), 375-394.

Fan, X., Blankenship, S.M., and Mattheis, J.P. (1999). 1-Methylcyclopropene inhibits apple ripening. *Journal of the American Society for Horticultural Science, 124*(6), 690-695.

FAOSTAT (2018). *FAOSTAT, Statistical data base*. Rome: Food and Agricultural Organizations of the United Nations. http://www.fao.org/faostat/en/#data. Retrieved 28th May 2019.

Fellman, J.K., Miller, T.W., Mattinson, D.S., and Mattheis, J.P. (2000). Factors that influence biosynthesis of volatile flavour compounds in apple fruits. *HortScience*, *35*(6), 1026-1033.

Fisher, F., and Applequist, D.E. (1965). Synthesis of 1-methylcyclopropene. *The Journal of Organic Chemistry*, *30*(6), 2089-2090.

Galil, J. (1968). An ancient technique for ripening sycamore fruit in East-Mediterranean countries. *Economic Botany*, *22*(2), 178-190.

Gerasopoulos, D., and Richardson, D.G. (1996). Effects of exogenous propylene and fruit calcium on ripening of non-chilled and chilled Anjou pears. *Postharvest Biology and Technology*, *8*(2), 111-120.

Giovannoni, J.J. (2001) Molecular biology of fruit maturation and ripening. *Annual Review of Plant Physiology and Plant Molecular Biology*, 52, 725–749.

Giovannoni, J.J. (2004) Genetic regulation of fruit development and ripening. *Plant Cell*, 16, S170–S180.

Giovannoni, J.J. (2008). Fruit ripening and its manipulation. In: *Annual Plant Reviews (Volume 26) Senescence Processes in Plants* (ed. Gan, S.). Blackwell Publishing Ltd, Oxford, UK, pp. 278-303.

Glowacz, M., Colgan, R., and Rees, D. (2015a). The use of ozone to extend the shelf-life and maintain quality of fresh produce. *Journal of the Science of Food and Agriculture*, *95*(4), 662-671.

Glowacz, M., Colgan, R., and Rees, D. (2015b). Influence of continuous exposure to gaseous ozone on the quality of red bell peppers, cucumbers and zucchini. *Postharvest Biology and Technology*, *99*, 1-8.

Gong, Y., Fan, X., and Mattheis, J.P. (2002). Responses of 'Bing' and 'Rainier' sweet cherries to ethylene and 1-methylcyclopropene. *Journal of the American Society for Horticultural Science, 127*(5), 831-835.

Gordon, M.S. (1980). Ring strain in cyclopropane, cyclopropene, silacyclopropane, and silacyclopropene. *Journal of the American Chemical Society, 102*(25), 7419-7422.

Grichko, V.P., Sisler, E.C., and Serek, M. (2003). Anti-ethylene properties of monoterpenes and some other naturally occurring compound in plants. *S aa S Bulletin. Biochemistry and Biotechnology, 16*, 20-27

Grierson, D. (1987) Senescence in fruits. *HortScience*, 22, 859–862.

Grierson, D. (2012). 100 years of ethylene—A personal view. In: *Annual Plant Reviews, The Plant Hormone Ethylene* (Volume 44) (ed. McManus, M.T.), Wiley-Blackwell Publishing, Ames, Iowa, USA, pp. 1–17.

Gross, K.C., Wang, C.Y., and Saltveit, M.E. (2016). *USDA Handbook 66: The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks*. pp. 792

Harker, F.R., Redgwell, R.J., Hallett, I.C., Murray, S.H., and Carter, G. (1997). Texture of fresh fruit. *Horticultural Reviews, 20*, 121-224.

Hill, A.G., and Rice, R.G. (1982). Historical background, properties and applications. In: *Handbook of Ozone Technology and Applications* (ed. Rice, R.G. and Netzer, A.), Ann Arbor Science Publication, Michigan, pp. 1-37.

Horvitz, S., and Cantalejo, M.J. (2014). Application of ozone for the postharvest treatment of fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, *54*(3), 312-339.

Huber, D.J., Jeong, J. and Mao, L.C. (2003). Softening during ripening of ethylene-treated fruits in response to 1-methylcyclopropene application. *Acta Horticulturae, 628*, 193-202.

Imaseki, H. (1991) The biochemistry of ethylene biosynthesis. In: *The Plant Hormone Ethylene* (eds. Mattoo, A.K. and Suttle, J.C.). CRC Publication Inc., Boca Raton, FL, pp. 1-21.

Jacobsen, J.V., and McGlasson, W.B. (1970). Ethylene production by autoclaved rubber injection caps used in biological systems. *Plant Physiology*, *45*(5), 631.

Jobling, J., Pradhan, R., Morris, S.C., Mitchell, L., and Rath, A.C. (2003). The effect of ReTain plant growth regulator [aminoethoxyvinylglycine (AVG)] on the postharvest storage life of' Tegan Blue' plums. *Australian Journal of Experimental Agriculture*, *43*(5), 515-518.

Johnson, D.S., and Ertan, U. (1983). Interaction of temperature and oxygen level on the respiration rate and storage quality of Idared apples. *The Journal of Horticultural Science*, *58*(4), 527-533.

Johnston, J.W., Gunaseelan, K., Pidakala, P., Wang, M., and Schaffer, R.J. (2009). Co-ordination of early and late ripening events in apples is regulated through differential sensitivities to ethylene. *Journal of Experimental Botany*, *60*(9), 2689-2699.

Johnston, J.W., Hewett, E.W., and Hertog, M.L. (2002). Postharvest softening of apple (*Malus domestica*) fruit: A review. *New Zealand Journal of Crop and Horticultural Science*, *30*(3), 145-160.

Juniper, B.E., Watkins, R., and Harris, S.A. (1998). The origin of the apple. *Acta Horticulturae*, *484*, 27-34.

Kader, A.A. (1980) Prevention of ripening in fruits by use of controlled atmospheres. *Food Technology*, *34*(3), 51-54.

Kader, A.A. (2001). Importance of fruits, nuts, and vegetables in human nutrition and health. *Perishables Handling Quarterly*, *106*(4), 6.

Kader, A.A. (2004). Increasing food availability by reducing postharvest losses of fresh produce. *Acta Horticulturae*. *682*, 2169-2176.

Kader, A.A., and Rolle, R.S. (2004). Postharvest treatments designed to manipulate the environment around produce in order to enhance quality. In: *The Role of Post-Harvest Management in Assuring the Quality and Safety of Horticultural Produce* (Vol. 152). Food and Agriculture Organisation, Rome. pp. 35-43.

Keller, N., Ducamp, M.N., Robert, D., and Keller, V. (2013). Ethylene removal and fresh product storage: a challenge at the frontiers of chemistry. Toward an approach by photocatalytic oxidation. *Chemical Reviews*, *113*(7), 5029-5070.

Khadre, M.A., Yousef, A.E., and Kim, J.G. (2001). Microbiological aspects of ozone applications in food: a review. *Journal of Food Science*, *66*(9), 1242-1252.

Khan, S.A.K.U. (2014). Regulation of postharvest life and quality in horticultural commodities at ambient conditions with new ethylene antagonists. *Doctoral Dissertation*, Curtin University, WA.

Kitinoja, L. (2013). *Use of Cold Chains for Reducing Food Losses in Developing Countries*. PEF White Paper No.13-03, Postharvest Education Foundation, La Pine, OR, USA.

Kitinoja, L., and Kader, A.A. (2002). *Small-Scale Postharvest Handling Practices: A Manual for Horticultural Crops*. University of California, Davis, Postharvest Technology Research and Information Center.

Kitinoja, L., Tokala, V.Y. and Brondy. A. (2018). A review of global postharvest loss assessments in plant-based food crops: Recent findings and measurement gaps. *Journal of Postharvest Technology*, 6(4), 1-15.

Klee, H.J. (2004). Ethylene signal transduction. Moving beyond Arabidopsis. *Plant Physiology*, *135*(2), 660-667.

Knee, M., Looney, N.E., Hatfield, S.G., and Smith, S.M. (1983). Initiation of rapid ethylene synthesis by apple and pear fruits in relation to storage temperature. *Journal of Experimental Botany*, *34*(9), 1207-1212.

Lapidge, S. (2015). *Primary Production Food Losses: Turning losses into profit. Primary Production Food Losses.* Turning losses into profit. Technical report. South Australian Research and Development Institute (PIRSA). Retrieved from: DOI: 10.13140/RG.2.1.1799.9525.

Larrigaudière, C., Vilaplana, R., Soria, Y., and Recasens, I. (2004). Oxidative behaviour of Blanquilla pears treated with 1-methylcyclopropene during cold storage. *Journal of the Science of Food and Agriculture, 84*(14), 1871-1877.

Larrigaudière, C., Vilaplana, R., Valentines, M.C., Soria, Y. and Recasens, I. (2005). Effects of 1-methylcyclopropene on antioxidant metabolism in cold- stored fruit. *Acta Horticulturae*, *682*, 957-964.

Łata, B., and Tomala, K. (2007). Apple peel as a contributor to whole fruit quantity of potentially healthful bioactive compounds. Cultivar and year implication. *Journal of Agricultural and Food Chemistry*, *55*(26), 10795-10802.

Leja, M., Mareczek, A., and Ben, J. (2003). Antioxidant properties of two apple cultivars during long-term storage. *Food Chemistry*, *80*(3), 303-307.

Levitzki, A. (1984). Receptors, a Quantitative Approach, Benjamin/Cummings, Menlo Park, Calif., pp. 91–101.

Li, J., Xiaoyu W., Honglin Y., Zonggan Y., Jiaxun W. and Yaguang L. (1989). Influence of discharge products on post-harvest physiology of fruit. In *Sixth International Symposium on High Voltage Engineering, New Orleans, LA, USA, August 28-September 1, 1989*, pp. 4.

Li, L., Lichter, A., Chalupowicz, D., Gamrasni, D., Goldberg, T., Nerya, O., Ben-Arie, R. and Porat, R. (2016). Effects of the ethylene-action inhibitor 1-methylcyclopropene on postharvest quality of non-climacteric fruit crops. *Postharvest Biology and Technology*, *111*, 322-329.

Lieberman, M. (1979) Biosynthesis and action of ethylene. *Annual Reviews of Plant Physiology*, *30*(1), 533-591.

Liew, C.L., and Prange, R.K. (1994). Effect of ozone and storage temperature on postharvest diseases and physiology of carrots (*Daucus carota* L.). *Journal of the American Society for Horticultural Science*, *119*(3), 563-567.

Lipinski, B., Hanson, C., Lomax, J., Kitinoja, L., Waite, R. and Searchinger, T. 2013. Reducing food loss and waste. Washington, DC: World Resources Institute. https://www.wri.org/publication/reducing-food-loss-and-waste Retrieved 28th May 2019.

Lurie, S., and Pesis, E. (1992). Effect of acetaldehyde and anaerobiosis as postharvest treatments on the quality of peaches and nectarines. *Postharvest Biology and Technology*, *1*(4), 317-326.

MacLean, D.D., Murr, D.P., DeEll, J.R., and Horvath, C.R. (2006). Postharvest variation in apple (*Malus*× *domestica* Borkh.) flavonoids following harvest, storage, and 1-MCP treatment. *Journal of Agricultural and Food Chemistry*, *54*(3), 870-878.

Maguire, K.M., Banks, N.H., and Opara, L.U. (2001). Factors affecting weight loss of apples. *Horticultural Reviews*, *25*, 197-234.

Mahajan, B.V.C., Kaur, T., Gill, M.I.S., Dhaliwal, H.S., Ghuman, B.S., and Chahil, B.S. (2010a). Studies on optimization of ripening techniques for banana. *Journal of Food Science and Technology*, *47*(3), 315-319.

Mahajan, B.V.C., Singh, K., and Dhillon, W.S. (2010b). Effect of 1-methylcyclopropene (1-MCP) on storage life and quality of pear fruits. *Journal of Food Science and Technology*, *47*(3), 351-354.

Malik, A.U., and Singh, Z. (2005). Pre-storage application of polyamines improves shelf-life and fruit quality of mango. *The Journal of Horticultural Science and Biotechnology*, *80*(3), 363-369.

Manganaris, G.A., Crisosto, C.H., Bremer, V., and Holcroft, D. (2008). Novel 1-methylcyclopropene immersion formulation extends shelf life of advanced

maturity ‘Joanna Red’ plums (*Prunus salicina* Lindell). *Postharvest Biology and Technology*, *47*(3), 429-433.

Manousaridis, G., Nerantzaki, A., Paleologos, E.K., Tsiotsias, A., Savvaidis, I.N., and Kontominas, M.G. (2005). Effect of ozone on microbial, chemical and sensory attributes of shucked mussels. *Food Microbiology*, *22*(1), 1-9.

Martínez-Romero, D., Bailén, G., Serrano, M., Guillén, F., Valverde, J.M., Zapata, P., Castillo, S. and Valero, D. (2007). Tools to maintain postharvest fruit and vegetable quality through the inhibition of ethylene action: a review. *Critical Reviews in Food Science and Nutrition*, *47*(6), 543-560.

Martínez-Romero, D., Dupille, E., Guillén, F., Valverde, J.M., Serrano, M., and Valero, D. (2003). 1-Methylcyclopropene increases storability and shelf life in climacteric and non-climacteric plums. *Journal of Agricultural and Food Chemistry*, *51*(16), 4680-4686.

Masia, A. (1998). Superoxide dismutase and catalase activities in apple fruit during ripening and post-harvest and with special reference to ethylene. *Physiologia Plantarum*, *104*(4), 668-672.

Mathooko, F.M., Tsunashima, Y., Kubo, Y., and Inaba, A. (2004). Expression of a 1-aminocyclopropane-1-carboxylate (ACC) oxidase gene in peach (*Prunus persica* L.) fruit in response to treatment with carbon dioxide and 1-methylcyclopropene: possible role of ethylene. *African Journal of Biotechnology*, *3*(10), 497-502.

Mathooko, F.M., Tsunashima, Y., Owino, W.Z., Kubo, Y., and Inaba, A. (2001). Regulation of genes encoding ethylene biosynthetic enzymes in peach (*Prunus persica* L.) fruit by carbon dioxide and 1-methylcyclopropene. *Postharvest Biology and Technology*, *21*(3), 265-281.

Mattheis, J.P. (2008). How 1-methylcyclopropene has altered the Washington State apple industry. *HortScience*, *43*(1), 99-101.

McMurchie, E.J., McGlasson, W.B., and Eaks, I.L. (1972). Treatment of fruit with propylene gives information about the biogenesis of ethylene. *Nature*, *237*(5352), 235.

Medlicott, A.P., Sigrist, J.M.M., Reynolds, S.B., and Thompson, A.K. (1987). Effects of ethylene and acetylene on mango fruit ripening. *Annals of Applied Biology*, *111*(2), 439-444.

Minas, I.S., Karaoglanidis, G.S., Manganaris, G.A., and Vasilakakis, M. (2010). Effect of ozone application during cold storage of kiwifruit on the development of stem-end rot caused by *Botrytis cinerea*. *Postharvest Biology and Technology*, *58*(3), 203-210.

Minas, I.S., Tanou, G., Belghazi, M., Job, D., Manganaris, G.A., Molassiotis, A., and Vasilakakis, M. (2012). Physiological and proteomic approaches to address the active role of ozone in kiwifruit post-harvest ripening. *Journal of Experimental Botany*, *63*(7), 2449-2464.

Mir, N.A., Curell, E., Khan, N., Whitaker, M., and Beaudry, R.M. (2001). Harvest maturity, storage temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Redchief Delicious' apples. *Journal of the American Society for Horticultural Science*, *126*(5), 618-624.

Müller, U.C., Bollig, M., and Peters, N. (1997). Approximations for burning velocities and Markstein numbers for lean hydrocarbon and methanol flames. *Combustion and Flame*, *108*(3), 349-356.

Musa, M.M.A. (2016). Structure activity relationship studies of new ethylene antagonists. *Doctoral Dissertation*, Curtin University, WA.

Nadas, A., Olmo, M., and Garcia, J.M. (2003). Growth of *Botrytis cinerea* and strawberry quality in ozone-enriched atmospheres. *Journal of Food Science*, *68*(5), 1798-1802.

Naeve L. (1997) Apple varieties and their uses. Horticulture and Home Pest News IC-477(23): 138–139.

Neljubov, D.N. (1901) Über die horizontale Nutation der Stengel von Pisum sativum und einiger anderen Pflanzen. *Pflanzen Beitrage und Botanik Zentralblatt* 10, 128–139 (Deutsch).

Ong, K.C., Cash, J.N., Zabik, M.J., Siddiq, M., and Jones, A. L. (1996). Chlorine and ozone washes for pesticide removal from apples and processed apple sauce. *Food Chemistry*, *55*(2), 153-160.

Ortuno Tomás, A., Garcia-Puig, D., Sabater, F., Porras, I., Garcia-Lidon, A. and Del Rio, J.A. (1993). Influence of ethylene and ethephon on the sesquiterpene nootkatone production in *Citrus paradisi*. *Journal of Agricultural and Food Chemistry*, *41*(10), 1566-1569.

Öztekin, S. (2018). Application of ozone as a postharvest treatment. *In Emerging Postharvest Treatment of Fruits and Vegetables* (eds. K. Barman, S. Sharma and M.W. Siddiqui), Apple Academic Press, USA, pp. 185-248.

Palou, L., Crisosto, C.H., Smilanick, J.L., Adaskaveg, J.E., and Zoffoli, J.P. (2002). Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. *Postharvest Biology and Technology*, *24*(1), 39-48.

Palou, L., Smilanick, J.L., Crisosto, C.H., and Mansour, M. (2001). Effect of gaseous ozone exposure on the development of green and blue molds on cold-stored citrus fruit. *Plant Disease*, *85*(6), 632-638.

Pandey, S., Ranade, S.A., Nagar, P.K., and Kumar, N. (2000). Role of polyamines and ethylene as modulators of plant senescence. *Journal of Biosciences*, *25*(3), 291-299.

Pathak, N., Caleb, O.J., Geyer, M., Herppich, W.B., Rauh, C., and Mahajan, P.V. (2017). Photocatalytic and photochemical oxidation of ethylene: potential for

storage of fresh produce—a review. *Food and Bioprocess Technology*, *10*(6), 982-1001.

Paull, R. (1999). Effect of temperature and relative humidity on fresh commodity quality. *Postharvest Biology and Technology*, *15*(3), 263-277.

Pell, E.J., Schlagnhaufer, C.D., and Arteca, R.N. (1997). Ozone-induced oxidative stress: mechanisms of action and reaction. *Physiologia Plantarum*, *100*(2), 264-273.

Pérez, A.G., Sanz, C., Ríos, J.J., Olias, R., and Olías, J.M. (1999). Effects of ozone treatment on postharvest strawberry quality. *Journal of Agricultural and Food Chemistry*, *47*(4), 1652-1656.

Pérez-Vicente, A., Martínez-Romero, D., Carbonell, A., Serrano, M., Riquelme, F., Guillén, F., and Valero, D. (2002). Role of polyamines in extending shelf life and the reduction of mechanical damage during plum (*Prunus salicina* Lindl.) storage. *Postharvest Biology and Technology*, *25*(1), 25-32.

Pesis, E. (2005). The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. *Postharvest Biology and Technology, 37*(1), 1-19.

Pesis, E., and Marinansky, R. (1992). Carbon dioxide and ethylene production by harvested grape berries in response to acetaldehyde and ethanol. *Journal of the American Society for Horticultural Science, 117*(1), 110-113.

Pirrung, M.C., Bleecker, A.B., Inoue, Y., Rodríguez, F.I., Sugawara, N., Wada, T., Zou, Y. and Binder, B.M. (2008). Ethylene receptor antagonists: strained alkenes are necessary but not sufficient. *Chemistry and Biology, 15*(4), 313-321.

Porat, R., Lichter, A., Terry, L.A., Harker, R., and Buzby, J. (2018). Postharvest losses of fruit and vegetables during retail and in consumers' homes: Quantifications, causes, and means of prevention. *Postharvest Biology and Technology*, 139, 135-149.

Pranamornkith, T., East, A., and Heyes, J. (2012). Influence of exogenous ethylene during refrigerated storage on storability and quality of *Actinidia chinensis* (cv. Hort16A). *Postharvest Biology and Technology*, *64*(1), 1-8.

Prasanna, V., Prabha, T.N., and Tharanathan, R.N. (2007). Fruit ripening phenomena–an overview. *Critical Reviews in Food Science and Nutrition*, *47*(1), 1-19.

Priyadarshani, A.M.B. (2017). A review on factors influencing bioaccessibility and bioefficacy of carotenoids. *Critical Reviews in Food Science and Nutrition*, *57*(8), 1710-1717.

Robles-Sánchez, R.M., Islas-Osuna, M.A., Astiazarán-García, H., Vazquez-Ortiz, F.A., Martín-Belloso, O., Gorinstein, S., and González-Aguilar, G.A. (2009). Quality index, consumer acceptability, bioactive compounds, and antioxidant activity of fresh-cut "Ataulfo" mangoes (*Mangifera indica* L.) as affected by low-temperature storage. *Journal of Food Science*, *74*(3), S126-S134.

Rodriguez, F.I., Esch, J.J., Hall, A.E., Binder, B.M., Schaller, E.G., and Bleecker, A.B. (1999) A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. *Science*, *283*, 396–398.

Rubin, M.B. (2001). The history of ozone. The Schönbein period, 1839–1868. *Bulletin for the History of Chemistry*, *26*(1), 40-56.

Sadler, G.D., and Murphy, P.A. (2010). pH and titratable acidity. In: *Food Analysis* (ed. Neilsen, S.). Springer, Boston, MA. pp. 219-238.

Saltveit, M.E. (1999). Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, 15(3), 279-292.

Saltveit, M.E. (2003). Is it possible to find an optimal controlled atmosphere? *Postharvest Biology and Technology*, *27*(1), 3-13.

Sarig, P., Zahavi, T., Zutkhi, Y., Yannai, S., Lisker, N., and Ben-Arie, R. (1996). Ozone for control of post-harvest decay of table grapes caused by *Rhizopus stolonifer*. *Physiological and Molecular Plant Pathology*, *48*(6), 403-415.

Schipperijn, A.J., and Smael, P. (1973). Chemistry of cyclopropene III: Synthesis of mono-and dialkylated cyclopropenes. *Recueil des Travaux Chimiques des Pays-Bas*, *92*(10), 1159-1166.

Schotsmans, W.C., Prange, R.K., and Binder, B.M. (2009). 1-Methylcyclopropene: mode of action and relevance in postharvest horticulture research. *Horticultural Reviews*, *35*, 263-313.

Scott, K.J., Yuen, C.M.C., and Ghahramani, F. (1995). Ethanol vapour—a new anti-scald treatment for apples. *Postharvest Biology and Technology*, *6*(3-4), 201-208.

Sease, W.S. (1976). Ozone mass transfer and contact systems. In: *Proceedings of The Second International Symposium on Ozone Technology.* International Ozone Institute, Syracuse, NY, pp. 1-14.

Selma, M.V., Ibáñez, A.M., Cantwell, M., and Suslow, T. (2008). Reduction by gaseous ozone of Salmonella and microbial flora associated with fresh-cut cantaloupe. *Food Microbiology*, *25*(4), 558-565.

Sfakiotakis, E.M., and Dilley, D.R. (1973). Induction of autocatalytic ethylene production in apple fruits by propylene in relation to maturity and oxygen. *Journal of the American Society for Horticultural Science*, *98*, 504–508.

Sfakiotakis, E.M., Stavroulakis, G., Ververidis, P., and Gerasopoulos, D. (1989). Induction of autocatalytic ethylene production and ripening by propylene in "Hayward" Kiwifruit. In: *Biochemical and Physiological Aspects of Ethylene Production in Lower and Higher Plants* (Eds. Clijsters, H., Proft, M. De, Marcelle, R., Van Poucke, M.) Springer, Dordrecht, pp. 173-178.

Sharma, S., Pareek, S., Sagar, N., Valero, D., and Serrano, M. (2017). Modulatory effects of exogenously applied polyamines on postharvest physiology, antioxidant system and shelf life of fruits: a review. *International Journal of Molecular Sciences*, *18*(8), 1789.

Singh, Z., Payne, A. D., Khan, S.A.K.U., and Musa, M.M. (2018). Method of retarding an ethylene response. *U.S. Patent Application No. 15/772,324*, filed November 15, 2018.

Sisler, E. C. (1979). Measurement of ethylene binding in plant tissue. *Plant Physiology*, *64*(4), 538-542.

Sisler, E.C. (2006). The discovery and development of compounds counteracting ethylene at the receptor level. *Biotechnology Advances*, *24*(4), 357-367.

Sisler, E.C. and Serek, M. (1997). Inhibitors of ethylene responses in plants at the receptor level: recent developments. *Physiologia Plantarum*, *100*(3), 577-582.

Sisler, E.C., Alwan, T., Goren, R., Serek, M., and Apelbaum, A. (2003). 1-Substituted cyclopropenes: effective blocking agents for ethylene action in plants. *Plant Growth Regulation*, *40*(3), 223-228.

Sisler, E.C., Blankenship, S.M., and Guest, M. (1990). Competition of cyclooctenes and cyclooctadienes for ethylene binding and activity in plants. *Plant Growth Regulation*, *9*(2), 157-164.

Sisler, E.C., Goren, R., and Huberman, M. (1985). Effect of 2, 5-norbornadiene on abscission and ethylene production in citrus leaf explants. *Physiologia Plantarum*, *63*(1), 114-120.

Sisler, E.C., Grichko, V.P., and Serek, M. (2006). Interaction of ethylene and other compounds with the ethylene receptor: agonists and antagonists. In: *Ethylene Action in Plants* (ed. Khan, N.A.). Springer, Berlin, Heidelberg, pp. 1-34.

Skog, L.J., and Chu, C.L. (2001). Effect of ozone on qualities of fruits and vegetables in cold storage. *Canadian Journal of Plant Science*, *81*(4), 773-778.

Skog, L.J., and Chu, C.L. (2003). Apples. In: *Encyclopedia of Food Science and Nutrition* (2nd Ed.) (eds. Caballero, B., Trugo, L.C. and Finglas, P.M.), Academic Press, Elsevier Science Ltd., Amsterdam, The Netherlands, pp. 290-294.

Solomos, T. (1983). Respiration and energy metabolism in senescing plant tissues. In: *Postharvest Physiology and Crop Preservation* (ed. Lieberman, M.). Springer, Boston, MA, pp. 61-98.

Song, J., Fan, L., Forney, C.F., Jordan, M.A., Hildebrand, P.D., Kalt, W., and Ryan, D.A.J. (2003). Effect of ozone treatment and controlled atmosphere storage on quality and phytochemicals in highbush blueberries. *Acta Horticulturae, 600*, 417-423.

Staby, G.L., Basel, R.M., Reid, M.S., and Dodge, L.L. (1993). Efficacies of commercial anti-ethylene products for fresh cut flowers. *HortTechnology*, *3*(2), 199-202.

Steinite, I., Gailite, A., and Ievinsh, G. (2004). Reactive oxygen and ethylene are involved in the regulation of regurgitant-induced responses in bean plants. *Journal of Plant Physiology*, *161*(2), 191.

Sugar, D. 2007. Postharvest handling of winter pears. In: *Pear Production and Handling Manual* (eds. E.J. Mitcham and R.B. Elkins). University of California Agriculture and Natural Resources Publication 3483, pp. 171–174.

Suwanagul, A., and Richardson, D.G. (1998). Identification of headspace volatile compounds from different pear (*Pyrus communis* L.) varieties. *Acta Horticulturae*. 475, 605-624.

Taiz, L., Zeiger, E., Møller, I.M., and Murphy, A. (2015) Ethylene: the gaseous hormone. In: *Plant Physiology and Development*. Sinauer Associates, Sunderland, MA, pp. 519-538.

Tavallali, V., and Moghadam, M.M. (2015). Postharvest application of AVG and 1-MCP enhance quality of ‘Kinnow' mandarin during cold storage. *International Journal of Farming and Allied Sciences, 4*(6), 526-535.

Thompson, A.K. (2010). Effects and interactions of CA storage. In: *Controlled Atmosphere Storage of Fruits and Vegetables*. CABI. pp. 11-26.

Tian, M.S., Hewett, E.W., and Lill, R.E. (1994). Effects of inhibitors on the carbon dioxide-stimulation of ethylene-forming enzyme activity in fruit of Japanese pear and apple. *Postharvest Biology and Technology*, *4*(1-2), 13-21.

Toivonen, P., and Lu, L. (2005). Studies on elevated temperature, short-term storage of 'Sunrise' summer apples using 1-MCP to maintain quality. *The Journal of Horticultural Science and Biotechnology*, *80*(4), 439-466.

Tokala, V.Y., Singh, Z., and Payne, A.D. (2018). Postharvest uses of ozone application in fresh horticultural produce. In: *Postharvest Biology and Nanotechnology* (eds. Paliyath, G., Subramanian, J., Lim, L-T, Subramanian, K.S., Handa, A. and Matoo, A.). John Wiley and Sons Inc., pp. 129-170.

Trinchero, G.D., Sozzi, G.O., Covatta, F., and Fraschina, A. A. (2004). Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of "Bartlett" pears. *Postharvest Biology and Technology*, *32*(2), 193-204.

Tucker, G.A. (2012). Introduction In. *Biochemistry of Fruit Ripening*. (eds Seymour, G., Taylor, J. and Tucker, G.). Springer, Dordrecht, Netherlands, pp. 1-51.

Tzortzakis, N. (2016). Ozone: a powerful tool for the fresh produce preservation In: *Postharvest Management Approaches for Maintaining Quality of Fresh Produce* (eds. Siddiqui, M.W.). Springer, Cham., pp. 175-207.

Tzortzakis, N., and Chrysargyris, A. (2017). Postharvest ozone application for the preservation of fruits and vegetables. *Food Reviews International*, *33*(3), 270-315.

Valero, D., Guillén, F., Valverde, J. M., Castillo, S., and Serrano, M. (2016). Recent developments of 1-methylcyclopropene (1-MCP) treatments on fruit quality attributes. In: *Eco-Friendly Technology for Postharvest Produce Quality* (ed. Siddiqui, M.W.), Academic Press, San Diego, CA. pp. 185-201.

Valero, D., Martínez-Romero, D., and Serrano, M. (2002). The role of polyamines in the improvement of the shelf life of fruit. *Trends in Food Science and Technology*, *13*(6-7), 228-234.

Van Buren, J.P. (1979). The chemistry of texture in fruits and vegetables. *Journal of Texture Studies*, *10*(1), 1-23.

Vilaplana, R., Valentines, M.C., Toivonen, P., and Larrigaudière, C. (2006). Antioxidant potential and peroxidative state of 'Golden Smoothee' apples treated with 1-methylcyclopropene. *Journal of the American Society for Horticultural Science*, *131*(1), 104-109.

Villalobos-Acuña, M.G., and Mitcham, E.J. (2008). Ripening of European pears: the chilling dilemma. *Postharvest Biology and Technology*, *49*(2), 187-200.

Villalobos-Acuña, M.G., Biasi, W.V., Flores, S., Jiang, C.Z., Reid, M.S., Willits, N.H., and Mitcham, E.J. (2011). Effect of maturity and cold storage on ethylene biosynthesis and ripening in 'Bartlett' pears treated after harvest with 1-MCP. *Postharvest Biology and Technology*, *59*(1), 1-9.

Vithana, M.D.K. (2017). Regulation of health-promoting compounds in ripe mango fruit. *Doctoral Dissertation*, Curtin University, WA. Retrieved from http://link.library.curtin.edu.au/p?cur_dspace_dc20.500.11937/66127 on 28th May 2019.

Vithana, M.D.K., Singh, Z., and Johnson, S.K. (2018). Dynamics in the concentrations of health-promoting compounds: lupeol, mangiferin and different phenolic acids during postharvest ripening of mango fruit. *Journal of the Science of Food and Agriculture*, *98*(4), 1460-1468.

Wang, B.G., Jiang, W.B., Liu, H. X., Lin, L., and Wang, J.H. (2006). Enhancing the post-harvest qualities of mango fruit by vacuum infiltration treatment with 1-methylcyclopropene. *The Journal of Horticultural Science and Biotechnology*, *81*(1), 163-167.

Wang, C.Y., Conway, W.S., Abbott, J.A., Kramer, G.F., and Sams, C.E. (1993). Postharvest infiltration of polyamines and calcium influences ethylene production and texture changes in Golden Delicious' apples. *Journal of the American Society for Horticultural Science*, *118*(6), 801-806.

Watkins, C.B. (2003). Principles and practices of postharvest handling and stress. In: *Apples: Botany, Production, and Uses* (eds. Ferree, I. and David, C.). CABI Publishing, MA, USA, pp. 585-614.

Watkins, C.B. (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances*, *24*(4), 389-409.

Watkins, C.B. (2008). Overview of 1-methylcyclopropene trials and uses for edible horticultural crops. *HortScience*, *43*(1), 86-94.

Watkins, C.B. (2015). Advances in the use of 1-MCP. In: *Advances in Postharvest Fruit and Vegetable Technology* (eds. Wills, R.B.H. and Golding, J.B.). CRC Press Boca Raton, pp. 117-145.

Watkins, C.B., Nock, J.F., and Whitaker, B.D. (2000). Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biology and Technology*, *19*(1), 17-32.

Wills, R.B.H., and Patterson, B.D. (1970). Ethylene, a plant hormone from fluorescent lighting. *Nature*, *225*, 199.

Wills, R.B.H., Warton, M.A., Mussa, D.M.D.N., and Chew, L.P. (2001). Ripening of climacteric fruits initiated at low ethylene levels. *Australian Journal of Experimental Agriculture*, *41*(1), 89-92.

Xu, L. (1999). Use of ozone to improve the safety of fresh fruits and vegetables. *Food Technology, 53*, 58-61,63.

Yang, S.F., and Hoffman, N.E. (1984). Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology, 35*(1), 155-189.

Yaseen, T., Ricelli, A., Turan, B., Albanese, P., and D'onghia, A. M. (2015). Ozone for post-harvest treatment of apple fruits. *Phytopathologia Mediterranea*, *54*(1), 94-103.

Zaharah, S.S. (2011). Hormonal regulation of mango fruit ripening. *Doctoral Dissertation*, Curtin University, WA. Retrieved from https://espace.curtin.edu.au/handle/20.500.11937/2008 on 28th May 2019.

Zhang, H., Wu, J., Zhao, Z., and Wang, Z. (2018). Non-destructive firmness measurement of differently shaped pears with a dual-frequency index based on acoustic vibration. *Postharvest Biology and Technology*, *138*, 11-18.

Zhang, M. J., Jiang, Y. M., Jiang, W. B., and Liu, X. J. (2006). Regulation of ethylene synthesis of harvested banana fruit by 1-methylcyclopropene. *Food Technology and Biotechnology*, *44*(1), 111-115.

Zhang, X., Zhang, Z., Wang, L., Zhang, Z., Li, J., and Zhao, C. (2011). Impact of ozone on quality of strawberry during cold storage. *Frontiers of Agriculture in China*, *5*(3), 356-360.

ANNEXURE

List of chemicals used for experiment and observations

Chemical used	Chemical Formula (PubChem CID)	Molecular weight (g mol^{-1})	Manufacture
Titratable acidity			
Sodium hydroxide (97%)	NaOH (14798)	40	Ecolab
Phenolphthalein (1%) solution	$C_{20}H_{14}O_4$ (4764)	318.33	Thermo Fisher
Ascorbic acid			
Meta-phosphoric acid (~65% HPO_3) pieces	HPO_3 (3084658)	80	Sigma-Aldrich
EDTA (Ethylenediaminetetraacetic acid) (99%)	$C_{10}H_{16}N_2O_8$ (6049)	292.24	Sigma-Aldrich
Folin-Ciocalteu Reagent			Thermo Fisher
Total antioxidant capacity			
Methanol (99.9%) 0.2µ filtered HPLC grade	CH_3OH (887)	32.04	Sigma-Aldrich
Sodium fluoride (99%)	NaF (5235)	41.99	Fluka
DPPH (2,2-diphenyl-1-picrylhydrazyl)	$C_{18}H_{12}N_5O_6$ (15911)	394.32	Sigma-Aldrich
Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) (97%)	$C_{14}H_{18}O_4$ (40634)	250.29	Sigma-Aldrich
Total phenol			
Methanol (99.9%) 0.2µ filtered HPLC grade	CH_3OH (887)	32.04	Sigma-Aldrich
Folin-Ciocalteu Reagent			Thermo Fisher
Sodium carbonate anhydrous (99.9%)	Na_2CO_3 (10340)	105.99	Merck
Gallic acid (97.5-102.5%)	$(HO)_3C_6H_2COOH$ (370)	170.12	Sigma-Aldrich
Individual sugars standard			
D-Fructose (99.95%)	$C_6H_{12}O_6$ (5984)	180.16	Sigma-Aldrich
D-Glucose (99.5%)	$C_6H_{12}O_6$ (5793)	180.16	Sigma-Aldrich
D-Sucrose (99%)	$C_{12}H_{22}O_{11}$ (5988)	342.30	Sigma-Aldrich
D-Sorbitol (99%)	$C_6H_{14}O_6$ (5780)	182.17	VMR
Organic acids standards			
Citric acid	$C_6H_8O_7$ (311)	192.12	Sigma-Aldrich
Malic acid	$C_4H_6O_5$ (525)	134.09	Sigma-Aldrich
Tartaric acid	$C_4H_6O_6$ (875)	150.09	Sigma-Aldrich

Fumaric acid	$C_4H_4O_4$ (444972)	116.07	Sigma-Aldrich
Succinic acid	$C_4H_6O_4$ (1110)	118.09	Sigma-Aldrich
Other chemicals			
Soda lime (4-8 mesh)	CaO. NaOH (66545795)	96.07	Thermo Fisher
Ethanol (99%)	C_2H_5OH (702)	46.07	Thermo Fisher

EcoLab: EcoLab Pty. Limited, New South Wales, Australia.
Thermo Fisher: Thermo Fisher Scientific Australia Pty. Limited, New South Wales, Australia.
Sigma-Aldrich: Sigma-Aldrich Pty. Limited, Steinheim, Germany.
Merck: Merck Pty. Limited, Victoria, Australia.
VMR: VMR Chemicals, BDH Laboratory supplies, England.

STATEMENT OF CONRIBUTION

To whom it may concern I, Vijay Yadav Tokala, contributed in designing and conducting the experiment, collection, analysing and interpretation of data and preparing the manuscripts in consultation with Prof. Zora Singh and Dr Alan D. Payne. Ms Poe Nandar Kyaw has assisted me in conducting experiments, biochemical analysis and recording data. I intend to be published following papers the with titles:

1. Vijay Yadav Tokala, Zora Singh, Alan D. Payne and Poe Nandar Kyaw. Fumigation and dip treatments of 1*H*-cyclopropabenzene and 1*H*-cyclopropa[*b*]naphthalene suppresses ethylene production and maintain fruit quality of cold stored 'Cripps Pink' apple
2. Vijay Yadav Tokala, Zora Singh, Alan D. Payne and Poe Nandar Kyaw. Effect of 1*H*-cyclopropabenzene and 1*H*-cyclopropa[*b*]naphthalene on ethylene production and fruit quality of 'Cripps Pink' and 'Granny Smith' apple stored in ozonized cold storage
3. Vijay Yadav Tokala, Zora Singh, Alan D. Payne and Poe Nandar Kyaw. Regulation of controlled atmosphere storage and fruit quality by ethylene antagonist fumigation and Airofresh® in Cripps Pink apples
4. Vijay Yadav Tokala, Zora Singh, Alan D. Payne and Poe Nandar Kyaw. Regulation of ethylene production and fruit quality by 1*H*-cyclopropabenzene and 1*H*-cyclopropa[*b*]naphthalene in controlled atmosphere stored Gold Rush pears
5. Vijay Yadav Tokala, Zora Singh, Alan D. Payne and Poe Nandar Kyaw. Effect of 1*H*-cyclopropabenzene and 1*H*-cyclopropa[*b*]naphthalene on postharvest physiology and fruit quality of controlled atmosphere stored Granny Smith apples
6. Vijay Yadav Tokala, Zora Singh, Alan D. Payne and Poe Nandar Kyaw. Regulation of ethylene action and fruit quality by 1*H*-cyclopropabenzene and 1*H*-cyclopropa[*b*]naphthalene in Cripps Pink apples stored in controlled atmosphere storage with AiroFresh®

VIJAY YADAV TOKALA

I, as a co-author, endorse that this level of contribution by the candidate indicated above is appropriate.

Co-author	Signature
1) **ZORA SINGH**	
2) **ALAN D. PAYNE**	
3) **POE NANDAR KYAW**