School of Molecular and Life Sciences

Regulation of Ethylene Production and Postharvest Fruit Quality of Stone Fruit Using Different Formulations of New Ethylene Antagonists

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Doctor of Philosophy

of

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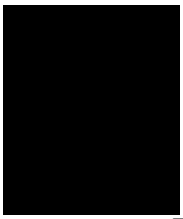
Declaration

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: 23.9.2019

Dedication

To:

My Beloved Parents,

U Kyaw Kyaw and Daw Pyone Pyone Maw;

My sister,

Ma Phyo Thinzar Kyaw;

My brother,

Mg Chann Myae Kyaw Kyaw;

For

"Their everlasting love and constant motivation throughout my PhD study…"

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The following work has been presented:

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Abstract

Stone fruits are one of the leading commercial fresh fruit in Australia for both domestic and export markets. Nonetheless, high ethylene production nature triggers rapid fruit ripening and softening limiting storage life and market potential. Without appropriate postharvest management, the stone fruit tends to over ripen, soften and lose their aesthetic and sensory qualities a week after harvest. Although 1-MCP is commercially available to suppress ethylene production, its application is still limited to places with the proper facilities and it is not available as pre-harvest treatment. The development of alternative ethylene antagonists which can overcome the limitations of 1-MCP would be beneficial to the stone fruit industry. New ethylene antagonists 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) have been proposed to retard ethylene actions in plants when applied as fumigants and prolong the storage life of fruit. They have the potential to be formulated as solutions. Aqueous formulations of these compounds would facilitate either dip or spray applications along the stone fruit supply chain. This thesis was undertaken to investigate the effects of different formulations of BC and NC on ethylene production and ripening associated quality changes of different stone fruit cultivars under storage conditions practised by the industry, i.e. ambient conditions, cold storage or modified atmosphere packaging. Quality parameters measured included fruit firmness, physiological weight loss, soluble solids content (SSC), titratable acidity (TA), SSC:TA, ascorbic acids, individual sugars and organic acids, total phenols, total anthocyanins and total antioxidant capacity.

Firstly, effects of different formulations of BC were evaluated on ‘Fortune’, ‘Angeleno’ and ‘Tegan Blue’ plums stored for 25 and 40 days at 0±1°C (90±5% relative humidity (RH)) to screen for the most effective formulations of BC. The fruit quality parameters responded differently to the BC formulations depending on the cultivars and the adjuvants applied. In all the tested plum cultivars, the BC fumigation exhibited the significantly ($P \leq 0.05$) highest ethylene suppression, followed by aqueous solutions of BC prepared with ethanol and with Tween® 20 in both storage periods.

Secondly, effects of different formulations of NC were evaluated on ‘Angeleno’ and ‘Tegan Blue’ plums stored for 25 and 40 days at 0±1°C (90±5%RH) to assess the most

effective formulations of NC. In both tested plum cultivars, NC formulations substantially reduced and delayed the ethylene production irrespective of the adjuvants applied in both storage periods. The effectiveness of NC formulations on the fruit quality parameters measured was influenced by the cultivars and the adjuvants applied. NC fumigation, NC aqueous solutions with ethanol and NC aqueous solution with Tween® 20 showed the greatest reduction in ethylene production compared to the control and other formulations.

Thirdly, effects of the most promising formulations of BC and NC were evaluated on three different stone fruits; 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum under ambient condition (at 20±1°C with 85±5% RH) for 10 days or under cold storage (0±1°C with 90±5% relative humidity) for 25 days. Regardless of fruit species, ethylene production and weight loss were significantly ($P \leq 0.05$) reduced in fumigation treatments with BC and NC, and firmness was also maintained. The effectiveness of aqueous solutions of BC and NC on ethylene production and quality parameters varied depending on the species, storage conditions and adjuvants applied. BC and NC aqueous solutions with the presence of ethanol were the best formulations for suppression of ethylene production in all the tested stone fruits.

The influence of different concentrations of ethanol on performance of aqueous solutions of BC and NC was also evaluated on 'Ruby Diamond' nectarine and 'Tegan Blue' plum under ambient conditions at 20±1°C with 85±5 % relative humidity for 10 days or under cold storage at 0±1 °C with 90±5% relative humidity for 25 days. The ethanol concentrations, 2.5 or 5.0 %, were found to enhance the performance of aqueous solutions of BC and NC in suppressing ethylene production of both the nectarine and plum cultivar. The quality parameters of the tested nectarine and plum responded differently depending on the concentration of ethanol.

The effects of ethylene antagonists (BC, NC and 1-methylcyclopropene (1-MCP)), applied either as fumigations or as aqueous solutions with ethanol, were investigated with or without modified atmosphere packaging (MAP) for cold storage life extension and fruit quality preservation of 'Angeleno' plum and 'Flavor Fall' pluot. The ethylene antagonists significantly ($P \leq 0.05$) suppressed ethylene production of plum and pluot regardless of MAP. The effect of aqueous solutions were more pronounced in MAP-stored fruit. With the application of ethylene antagonists and MAP, 'Angeleno' plum

and ‘Flavor Fall’ pluot can be stored up to 50 and 60 days respectively under cold storage (0±1°C and 90±5% RH) preserving the fruit quality.

Overall, BC and NC fumigations and spray solutions with ethanol were found to be the most effective formulations, compared to control, to regulate ethylene production and to maintain fruit quality of the tested stone fruits during different postharvest storage conditions. The discovery of an effective spray solution would allow BC and NC to be applied in open field conditions in the future.

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List of symbols and abbreviations

$	Dollar
x	Multiply/ interaction
≤	Less than or equal
≥	Greater than or equal
≈	Approximately
±	Plus or minus
%	Percentage
/	Divide/ or
&	And
β	Beta
α	Alpha
γ	Gamma
λ	Wavelength
™	Trade mark
°C	Degrees Celsius
µL	Micro Litre
µmol	Micro Mole
µM	Micro molar
1-MCP	1-methylcyclopropene
2,5-NBD	2,5 Norbornadiene
atm	Atmosphere
Å	Angstrom unit (10^{-8} centimetre)
ABS	Australian Bureau of Statistics
ACC	1-aminocyclopropane-1-Carboxylic Acid
ACO	1-aminocyclopropane-1-Carboxylic Acid Oxidase
ACS	1-aminocyclopropane-1-Carboxylic Acid Synthase
ATP	Adenosine Triphosphate
AVG	1-Aminoethoxyvinylglycine
BC	1 *H*-cyclopropabenzene
cm	Centi Metre
cv	Cultivar(s)
CA	Controlled atmosphere

C_2H_4	Ethylene
CO_2	Carbondioxide
Co^{2+}	Cobalt II ion
CPAS	3-cyclopropyl-1-enyl-propanoic acid sodium salt
dH_2O	Distilled water
DPPH	2,2-diphenyl-1-picrylhydrazyl
et al.	et alia
ETD	Ethylene detector
ETR	Ethylene response
EIN	Ethylene insensitive
ER	Endoplasmic reticulum
ERS	Ethylene response sensor
FAO	Food and Agriculture Organization
Fig.	Figure
g	Relative centrifugal force / G-Force
g	Gram
GAE	Gallic acid equivalent
GC	Gas Chromatogram
h	Hour
HPLC	High performance liquid chromatography
kPa	Kilo Pascal
mL	Milli Litre
mM	Milli Molar
mm	Millimeter
M	Molarity
MAP	Modified atmosphere packaging
Min	Minutes
L	Litre
LSD	Least significant difference
MT	Metric Tonnes
Ni^{2+}	Nickel II ion
N	Normality/ Newton(s)
N_2	Nitrogen

NaOH	Sodium hydroxide
NaF	Sodium fluoride
NC	1 *H*-cyclopropa[*b*]naphthalene
NSW	New South Wales
O_2	Oxygen
P	Probability
PG	Polygalacturonase
ppm	Parts per million
QLD	Queensland
rpm	revolutions per minute
RH	Relative humidity
s	second
SA	South Australia
SAM	*S*-adenosyl methionine
SE	Standard error
SSC	Soluble solids content
TA	Titratable acidity
TAS	Tasmania
TEAC	Trolox equivalent antioxidant activity
UK	United Kingdom
USA	The United States of America
USDA	United States Department of Agriculture
UV	Ultra-Violet
v/v	Volume by volume
vol.	volume
VIC	Victoria
w/v	Weight by volume
WA	Western Australia

CHAPTER 1

General introduction

The growing world population, which is estimated to be 9 billion by 2050, is becoming a global challenge to food security (Godfray et al., 2010). However, only two-thirds of the food produced reach the consumer and the other one third is being wasted or lost, according to the Food and Agriculture Organization of the United Nations (FAO, 2011). A report by the Department of the Environment and Energy estimated that the food wasted worldwide each year amounts to $ 20 billion, this includes all the plant and animal-based food produced for human consumption (DEE, 2017). Reducing food loss and waste has been reported as one of the strategies to tackle the global threat to food security (Godfray et al., 2010). The reduction of food losses and waste has a flow-on effect considering the soil, water, energy and the human labour resources which are invested for the food production (FAO, 2018). Papargyropoulou et al., (2014) suggested that prevention of the food losses along the supply chain through technological improvements of the storage life is the most favourable option to address food wastage.

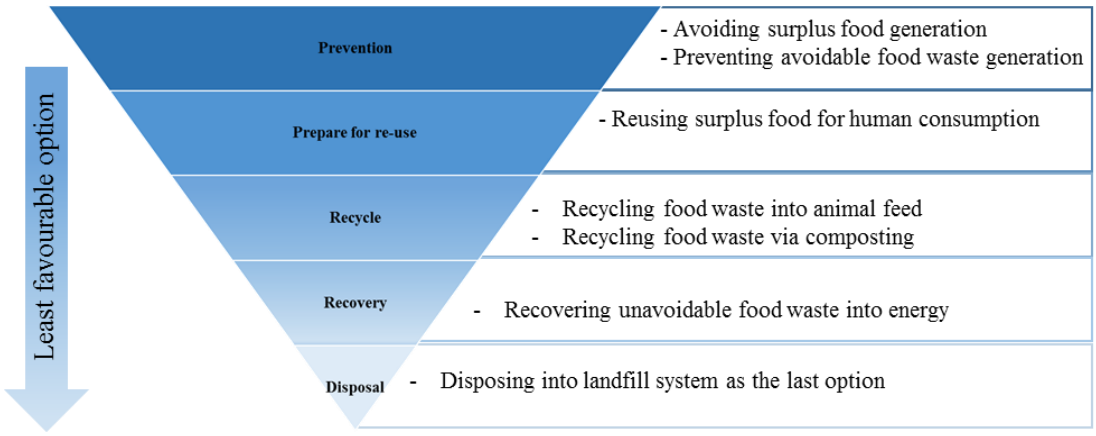


Fig. 1.1 The food waste management hierarchy modified from Papargyropoulou et al., (2014).

From the perspective of the horticulture industry, fruits and vegetables are highly vulnerable to the losses due to their easily perishable nature, especially at harvest and during postharvest handling. A case study on the postharvest losses along the domestic tomato supply chain in Queensland reported that 40.3-55.9 % of the total harvestable tomato crop was lost before reaching the consumer, which included both the damaged

and undamaged tomatoes (McKenzie et al., 2017). Considering the damaged tomatoes, it was identified that almost 50 % at harvest and 39 % at the packing shed were due to over-ripening. In Australia, the cost of losses in fruit and vegetables to the primary producer is seven times more than the cost of losses in cereal crops (Lapidge, 2015).

The summer fruits, including stone fruits (peach, nectarine and plum) are the 6th largest commercial fruit crop in Australia (Horticulture Innovation Australia Limited, 2017). In terms of export volume (intonnes), they hold the 4th largest among the exporting fruit crops after citrus, table grapes and melons. The majority of total peach/nectarine (91%) and plum (69 %) harvested are supplied as fresh either for the domestic or the export market (Horticulture Innovation Australia Limited, 2017). However, like other horticultural crops, they are highly vulnerable to the postharvest losses along the supply chain. The delicate thick flesh of stone fruit is prone to softening making them susceptible to physical damage and biotic attacks during ripening (Crisosto et al., 2009). Moreover, they are climacteric fruits with a large increase in ethylene production during fruit ripening, which promotes the subsequent ripening related fruit quality changes (Crisosto and Day, 2012). The rapid fruit ripening and perishable nature of stone fruit limit the storage life leading to the reduction of the market potential and the economic losses of the grower. The oversupply of the fruit during the harvest period also leads to wastage without reaching the consumer (Crisosto et al., 1995; Khan et al., 2018).

Ethylene is the main natural cause to the spoilage of horticultural crops as it initiates ripening of the fruits, wilting of vegetables and abscission of flowers (Kader, 1985). It is responsible for the irreversible changes in fruit quality attributes related to fruit ripening such as colour development, fruit softening, sugar accumulation and flavour/ aroma developments (Bapat et al., 2010). Controlling the presence of ethylene along the supply chain of the fruits and vegetables has been recommended as one of the key strategies not only to postpone fruit ripening and to preserve quality deterioration but to prolong storage life and to reduce food wastage (Blanke, 2014; Zhang et al., 2017; Khan et al., 2018). A number of practices have been used to prevent the harmful effect of ethylene during the storage of fresh fruits. These practices include controlling storage temperature and atmosphere, inhibiting ethylene biosynthesis, removing

ethylene present in the storage atmosphere and blocking the perception of ethylene at the receptor level (Keller et al., 2013; Blanke, 2014; Zhang et al., 2017).

Controlling the storage temperature and the storage atmosphere are the postharvest strategies which indirectly control ethylene production by reducing respiration rate of fresh fruits. As a result of the reduction in the amount of respiratory product ATP, which is the rate-limiting factor in the conversion of methionine (MET) to *S*-adenosylmethionine (SAM), the biosynthesis of ethylene is limited (Taiz and Zeiger, 2006; Blanke, 2014; Zhang et al., 2017). Storage at low temperature has been a fundamental practice for storage life extension and quality preservation of stone fruit as it can slow down the metabolic processes of fruits (Crisosto et al., 1995; Valero and Serrano, 2010a). Modified atmosphere packaging (MAP) is applied as a complement postharvest management practise to the cold storage technology for delayed fruit ripening, reduced water loss and disease development during the storage of stone fruit (Crisosto et al., 2009). The lower oxygen and higher carbon dioxide atmosphere inside the MAP can further suppress respiration rate of fruit and the subsequent metabolic processes (Brandenburg and Zagory, 2009).

1-Methylcyclopropene (1-MCP) is an ethylene antagonist which is used to mitigate the ill-effects of ethylene in fresh fruits (Blankeship and Dole, 2003; Watkins, 2015). 1-MCP blocks the perception of ethylene at the receptor level and thus the signalling pathway of ethylene is inhibited (Sisler, 2006). A few other antagonists have been reported but have not seen wide usage in the literature. The compounds 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) could provide an alternative to 1-MCP in retarding the ethylene actions in plants (Singh et al., 2018). Although BC and NC possess different molecular structures to 1-MCP, their chemical reactivities are similar to that of 1-MCP which is proposed to proceed via a ring-opening mechanism to irreversibly bind to the ethylene receptor (Pirrung et al., 2008). BC and NC, being liquid and solid respectively at room temperature, are relatively easier to handle as compared to the gaseous compound 1-MCP. These partially water soluble hydrocarbons may have the potential to be formulated as aqueous solutions. *The use of aqueous solutions BC and NC to antagonize the ethylene action is yet to be investigated.*

The application of adjuvants in preparation of aqueous agrochemicals is a common practice to increase the solubility and the permeability of active compounds via surface contact (Somervaille et al., 2012). Co-solvents, especially alcohols can increase the solubility of hydrocarbons (Grichko, 2006) and can enhance the permeability of lipid membranes (Patra et al., 2006). The addition of surfactants increases the infiltration of active compounds by altering the permeability of cuticle and by increasing the surface tension (Harker and Ferguson, 1991; Singh and Khan, 2012). Cyclodextrins are used as a carrier compound in different commercial formulations of 1-MCP to increase the solubility and to permit the slow release of 1-MCP (Watkins, 2015). The addition of adjuvants is likely to increase the solubility and the performance of the aqueous solutions of the ethylene antagonists BC and NC.

Project aims:

The development of aqueous formulations of BC and NC will ensure the ease in application and availability to all the stakeholders along the stone fruit supply chain. It would allow user-friendly methods, such as spray or dip treatments, to be used along the packing-lines during postharvest handling or on display shelves of the retail outlets. Aqueous solutions of BC and NC would be applicable in places where the proper facilities for fumigation treatment are not available and in pre-harvest applications. Therefore, the main objective of this thesis was to investigate the formulations of 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) which would be practical for the stone fruit industry. The effectiveness of different formulations of BC and NC, with or without adjuvants, were examined under various standard storage conditions practised by the stone fruit industry.

The following specific aims were set to address the main objective of the study:

(1) To identify the most effective formulations of BC in retarding ethylene production and maintaining fruit quality of ‘Fortune’, ‘Tegan Blue’ and ‘Angeleno’ plums under cold storage (0±1 °C and 90±5 % RH), which is the storage condition practically used in the industry during storage and transportation of stone fruit.

(2) To identify the most effective formulations of NC in retarding ethylene production and maintaining fruit quality of ‘Tegan Blue’ and ‘Angeleno’ plums under cold storage (0±1 °C and 90±5 % RH).

(3) To investigate and optimise the effectiveness of the best formulations of BC and NC in retarding ethylene production and maintaining fruit quality of different stone fruit species peach, nectarine and plum under cold storage.

(4) To optimise the concentrations of the best adjuvant, ethanol, in order to improve the performance of aqueous solutions of BC and NC in retarding the ethylene production and preserving the fruit quality.

(5) To investigate the synergistic effect of BC and NC and modified atmosphere packaging (MAP) compared to commercially available ethylene antagonist (1-MCP) to extend the cold storage life of ‘Angeleno’ plum and ‘Flavor Fall’ pluot.

CHAPTER 2

General literature review

2.1 Introduction

2.1.1 Stone fruits: Peach, nectarine, plum and pluot

Stone fruits, which are named after their hard seed-coat, are economically important crops as fresh fruit, processed fruit or nuts throughout the world. They belong to the family Rosaceae and more specifically the genus *Prunus* (Hummer and Janick, 2009). Within the genus *Prunus*, peaches, nectarines, plums, cherries and apricots hold the greatest market potential in fruit industry because of their attractive flavours and health-promoting nutritive values (Crisosto and Day, 2012).

Peaches (*Prunus persica* L. Batsch) and nectarines (*Prunus persica* var. nucipersica) share the largest proportion of the stone fruit market. They are native to China where stone fruit cultivation has the longest history around the world. Nectarines are the mutants of peaches without fuzzy skin. Depending on the flesh types, peaches and nectarines are classified into two groups: freestone (melting) and clingstone (non-melting or rubbery). The melting type varieties are primarily for fresh fruit consumption while the non-melting types are for processing. The non-melting types are also becoming popular in the fresh market due to their firm flesh which is beneficial for prolonged storage and distance shipping (Hummer and Janick, 2009; Crisosto and Day, 2012).

Plums are also economically important stone fruits around the world with a diverse group of commercial cultivars. The two major economically important plums are Japanese plums (*P. salicina* L.) which are mainly marketed for fresh consumption and European plums (*P. domestica* L.) which are mostly known for processing (Khan et al., 2018). Plums are famous for their richness in polyphenols and anthocyanins, which are their main sources of antioxidant properties, and have the potential to be included as a functional food in the human diet (Sahamishirazi et al., 2017). Although antioxidant properties may not directly correlate to a healthier product (Bast and Haenen, 2013), it is a good marketing tool.

The development of new interspecific *Prunus* varieties such as pluots, plumcots, and apriums with more desirable characteristics such as intense flavour, high sweetness, firm flesh and high antioxidant levels have expanded the market share of stone fruit in the fruit industry (Crisosto et al., 2007; Hummer and Janick, 2009). Pluots are the result of complex interspecific crosses between plum and apricot with a greater percentage of plum character. They possess notable sensory characteristics such as strong plum flavour, high sugar content and antioxidant activity (Crisosto et al., 2007). Some of the pluot cultivars such as 'Flavor Fall' [(*P. salicina* x (*P. salicina* x *P. armeniaca*)] possess long storage life up to 42 days (Duvenhage et al., 2012), more desirable shipping qualities such as firmer flesh and late maturity (Zaiger et al., 2001) which are attractive to growers and postharvest handlers.

2.1.2 Production Statistics of stone fruits in Australia

Stone fruits play a major role in the Australian fruit industry and are being produced throughout Australia (Fig. 2.1). Peaches, nectarines and plums are classified as summer fruit due to their availability during the summer fruit season (October to April). They constitute the majority (more than 90%) of summer fruit production in Australia (Fig. 2.2). Within Australia, Victoria is the major summer fruit producer which holds two-thirds of total production followed by New South Wales, Western Australia, South Australia, Queensland and Tasmania (Fig. 2.3). According to the Australian Bureau of Statistics (ABS, 2018), annual stone fruit production is ≈1.2 million tonnes with the total wholesale value of $370m. Australian summer fruits are mainly exported to Asia (68 % of total export) and the Middle East (29 %) (Horticulture Innovation Australia Limited, 2017). In the world production context, almost half of the world production is contributed by China which has the largest and longest history of stone fruit production, followed by Spain, Italy and USA (FAO, 2017).

2.1.3 Limitations in the stone fruit industry

Despite the attractive flavour and nutritional profiles of stone fruit, fresh peach, nectarine and plum are extremely perishable and have very short marketable life. Their inherent fruit physiology, the extreme sensitivity to the natural ripening hormone 'ethylene', is the main cause of these limitations. They are climacteric fruits which

exhibit a rise in ethylene production at the commencement of ripening. This ethylene climacteric peak accelerates senescence processes and leads to the postharvest quantity and quality losses (Crisosto et al., 2007). Without effective postharvest management, they tend to over ripen within a week after harvest. The short stone fruit harvest season creates the massive fruit supply in the market which reduces profitability to the growers by the economics of supply and demand. The present review will cover the ripening physiology of stone fruits and the research conducted on the postharvest management of stone fruits. This will help us uncover the research gaps that need to be investigated to further improve the management of stone fruits in order to allow the highest profit to the growers and maximum availability of stone fruit to the consumers.

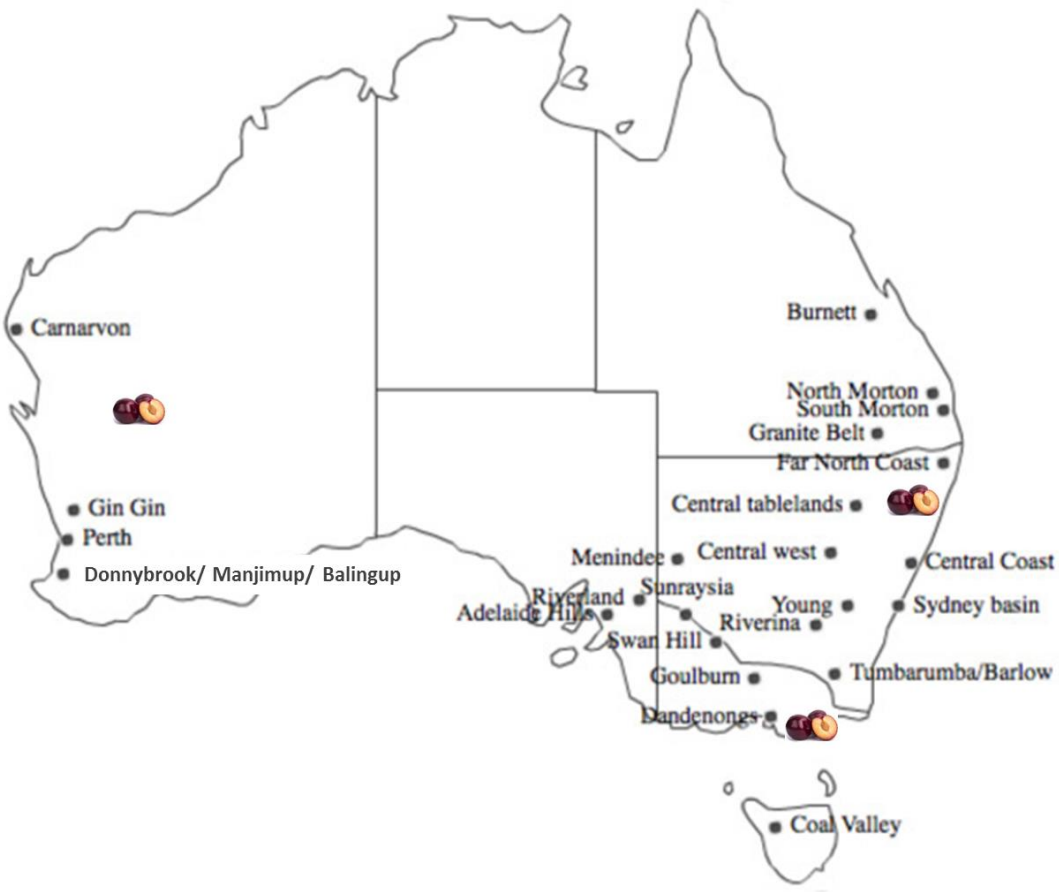


Fig. 2. 1 Major stone fruit production areas in Australia. (Source: http://www.australiafresh.com.au)

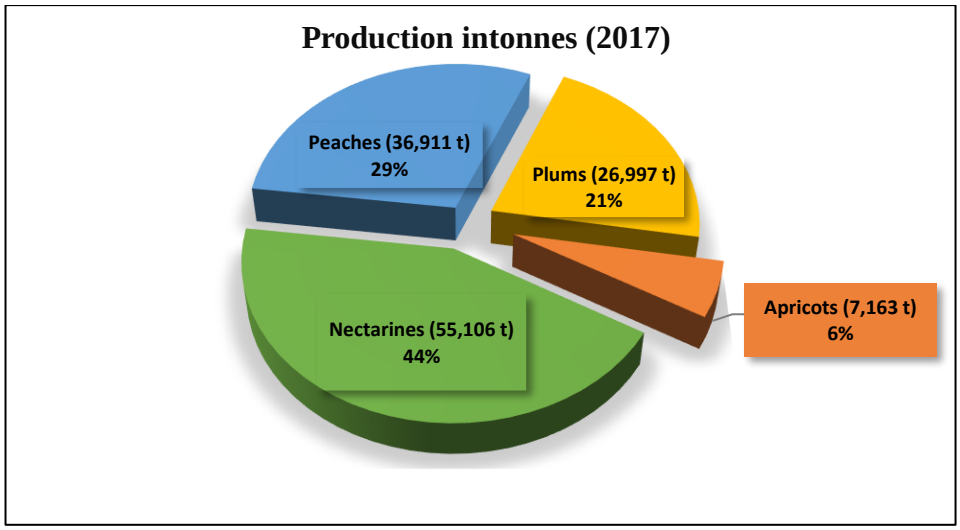


Fig. 2. 2 Summer fruit production in whole Australia. (Source: Horticulture Innovation Australia Limited, 2017).

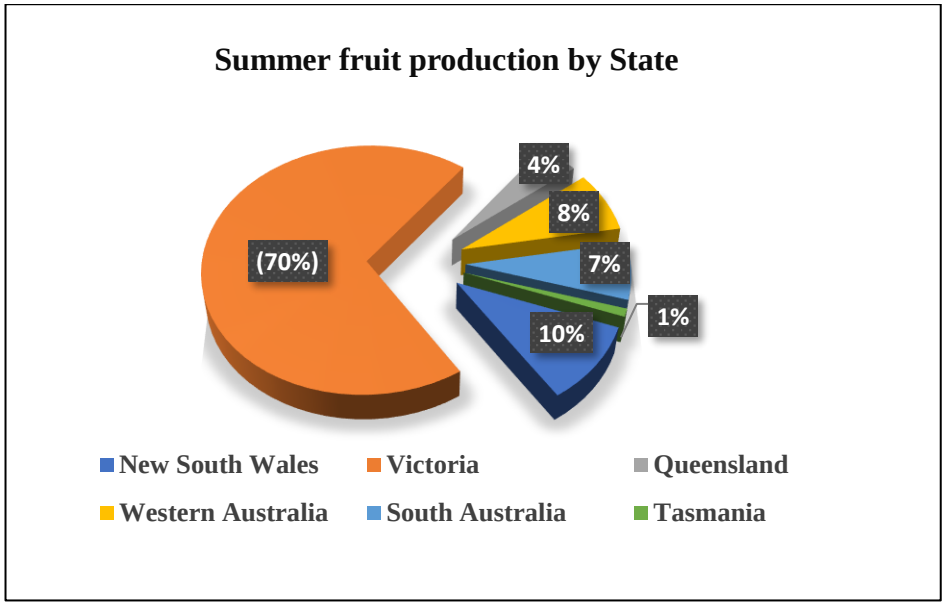


Fig. 2. 3 Total summer fruit production in each state of Australia. (Source: Horticulture Innovation Australia Limited, 2017).

2.2 Ethylene: A ripening promoter

2.2.1 Role of ethylene in fruit ripening

The role of ethylene in fruit ripening has been well recognised from more than half a century. Ethylene is a plant hormone that triggers ripening and the related processes in

climacteric fruit. However, it does not have a prominent role in non-climacteric fruit ripening (Burg and Burg, 1965; Pech et al., 2012). In climacteric fruit such as peaches, nectarines and plums, a burst of ethylene production occurs at the onset of ripening and this ethylene signals the subsequent changes of ripening such as fruit softening (Khan and Singh, 2007a), sugars accumulation (Singh and Singh, 2008), chlorophyll degradation (Rozo-Romero et al., 2015) and development of fruit flavour and aroma compounds (Pech et al., 2012). This climacteric rise in ethylene may be observed prior to or after the respiratory climacteric peak has occurred. In contrast, non-climacteric fruit does not exhibit a sharp climacteric peak of ethylene. Their ethylene production is very low and constant throughout the ripening process. However, ethylene induces fruit softening and colour (carotenoids and anthocyanins) development in non-climacteric fruits like strawberry (Tian et al., 2000), citrus (Stewart and Wheaton, 1972; Mayuoni et al., 2011) and grape berries (El-Kereamy et al., 2003). The recent genomic studies confirmed the non-climacteric pattern of ethylene production in 'Santa Rosa' plum and 'Sweet Miriam' plum, which is the bud-sport mutant derived from 'Santa Rosa' (Fernandez i Marti et al., 2018 and Minas et al., 2015). 'Sweet Miriam' plum did not even respond to the external ethylene treatment with 500 µL L-1 of propylene, an ethylene inducer (Minas et al., 2015). In addition to climacteric and non-climacteric fruits, the suppressed-climacteric fruit such as 'Shiro', 'Rubyred' (Abdi et al., 1998) and 'Angeleno' plum (Candan et al., 2008) also respond to ethylene. The suppressed-climacteric fruit are those that are unable to produce a drastic amount of autocatalytic ethylene during ripening like normal climacteric fruit, but they respond to the exogenously applied ethylene (Abdi et al., 1997). According to Abdi et al. (1998), the suppressed-climacteric plums 'Shiro' and 'Rubyred' express 15-500 fold reduced ethylene production than the climacteric plums 'Gulfruby' and 'Beauty'. The suppressed-climacteric plum 'Angeleno' exhibits reduction in fruit firmness and titratable acidity content in response to the exogenous ethylene (Minas et al., 2015). The information of climacteric classification of the plum cultivars tested in this study, except 'Angeleno' which is phenotypically and genetically classified as suppressed-climacteric type (Minas et al., 2015), is still unknown and is warranted for future investigation.

2.2.2 Ethylene biosynthesis

Ethylene can be produced in almost all parts of the plant at different stages of development, however, the amount may vary depending on developmental stage and plant parts (Burg and Burg, 1962; Taiz and Zeiger, 2006). Ethylene production pathway starts with the transformation of amino acid methionine into *S*-adenosyl-methionine (SAM) which is mediated by SAM-synthetase. SAM is converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by the ACC synthase enzyme and, ACC breakdowns into ethylene in the presence of ACC oxidase enzyme. Simultaneously, methionine is being recycled through the Yang cycle for constant production of ethylene (Taiz and Zeiger, 2006; Nath et al., 2006). The ethylene production pathway and its action during fruit ripening is illustrated in Fig. 2.4.

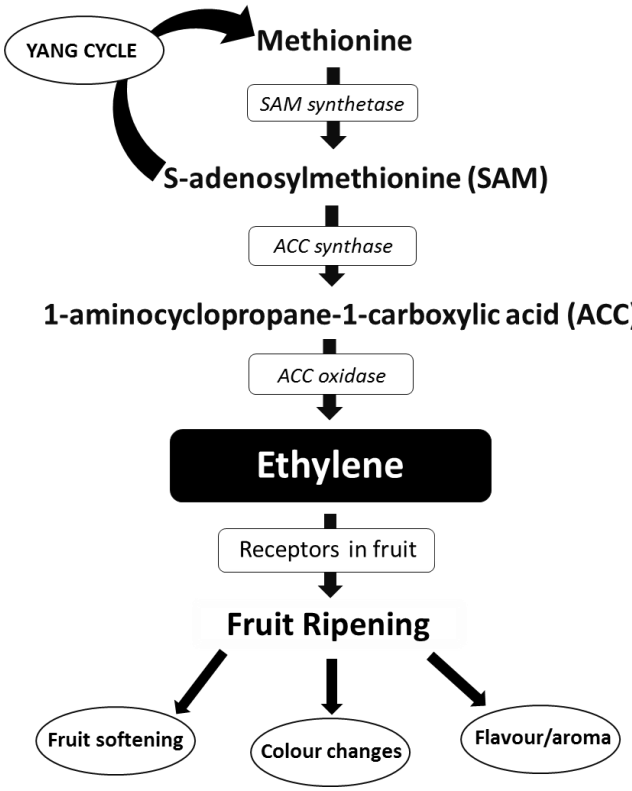


Fig. 2. 4 Ethylene biosynthesis pathway and actions in fruit

2.2.3 Ethylene perception and signal transduction

Being a gas, ethylene diffuses from the source of production to other parts of plant tissue. The response of plant tissue to ethylene is through the signal transduction

pathway described in Fig. 2.5 (Taiz and Zeiger, 2006). Ethylene receptors have a copper(I) cofactor and are located in the endoplasmic reticulum of the cell. Studies into *Arabidopsis* revealed a family of ethylene receptors which is composed of the subfamily 1 (ETR1 and ERS1) and subfamily 2 (ETR2, ERS2 and EIN4). These general features and the subfamilies of ethylene receptors are conserved in monocots and dicots (Hall et al., 2007). In general, the ethylene receptor has three main components: the ethylene binding site domain with the copper(I) cofactor, the histidine kinase or GAF domain which is spanned to the endoplasmic reticulum and the receiver domain which functions to pass the signal to the nucleus for cellular responses (Fig. 2.5) (Alonso and Stepanova, 2004; Hall et al., 2007). However, the histidine and receiver domains are not conserved in all the members of ethylene receptors. Studies on ethylene receptor genes of *Arabidopsis* revealed that a canonical histidine moiety is present only in ETR1 and ERS1, and the receiver domain is absent in ERS1 and ERS2 (Hua et al., 1998; Alonso and Stepanova, 2004). The number of ethylene receptors present and their actions may vary from species to species, even from cultivar to cultivar. It is assumed that new receptors can be synthesised after all the existing receptors are being blocked or permanently bound, however, there is little evidence (Schotsmans et al., 2009). The exact structure of ethylene receptors and their actions for each type of crop are still to be examined.

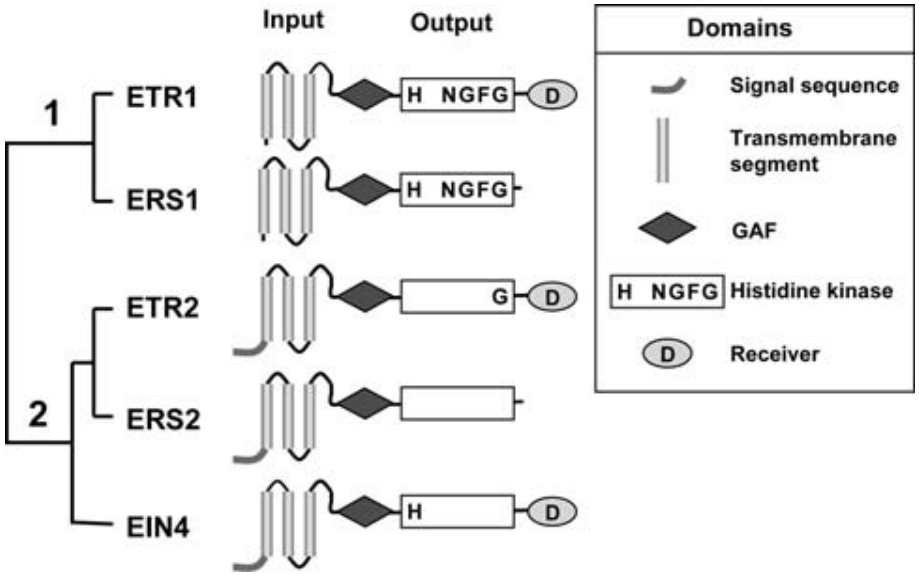


Fig. 2. 5 The general features of ethylene receptor family in the ethylene signaling pathway of *Arabidopsis.* (Hall et al., 2007).

2.2.4 Ethylene agonists

Ethylene agonists are compounds that bind to the receptors giving similar cellular response as ethylene. A number of alkenes and alkene-related compounds such as propylene, acetylene, 1-butyne have been demonstrated as ethylene agonists based on the similarity of their responses in pea seedling growth to ethylene (Burg and Burg, 1967; Sisler et al., 2006). The application of acetylene as a fruit ripening promoter was reported in mango (Medlicott et al., 1987) and banana (Thompson and Seymour, 1982). Propylene has also been used as ripening promoter in the studies of fruit physiology of plum (Abdi et al., 1997), tomato (McGlasson et al., 1975) and strawberry (Perkins-Veazie et al., 1996).

2.2.5 Ethylene antagonists: Inhibitors of ethylene actions

Ethylene antagonists are compounds that bind to the ethylene receptors but do not trigger a cellular response and block the action of ethylene in plants (Sisler and Serek, 1997 and 2003). Sisler (2006) has reviewed the stepwise development of compounds such as 2,5-norbornadiene (2,5-NBD), *trans*-cyclooctene, diazocyclopentadiene (DCPA), cylopropenes (CP) and substituted cyclopropenes (1-methylcyclopropene and 3,3-dimethylcyclopropene), which compete with ethylene at the receptor level as antagonists. Several different types of compounds such as 1-ethylcyclopropene and 1-propylcyclopropene (Feng et al., 2004), alkyl-2-cyclopropene-1-carboxylic acid ethyl ester (Choi, 2013), 1-(3-phenyl-propyl) cyclopropene (Song et al., 2018), *N*,*N*-dialkyl-(1-cyclopropenylmethyl)amine (Seglie et al., 2010) and 3-cyclopropyl-1-enyl-propanoic acid sodium salt (Goren et al., 2011), has also been documented as potential ethylene antagonists. Out of all the compounds, 1-MCP is the most promising ethylene antagonist which can be applied as a fumigant in ethylene-responsive plant parts (Sisler et al., 2006; Watkins, 2006).

2.2.5.1 1-Methylcyclopropene (1-MCP): Current commercial ethylene antagonist

The compound 1-MCP is a gaseous ethylene antagonist which blocks the receptor for a long period even at a considerably low concentration. It is highly active and stable as a gas at room temperature, however, it is unstable in the liquid state even at -20 °C

(Sisler and Serek, 1997). 1-MCP is commercially available at AgroFresh Inc. either as EthylBloc® which is mainly for ornamental crops or SmartFresh™ which is for edible crops. Commercial 1-MCP is formulated as powder and is a complex with γ-cyclodextrin. The powder releases 1-MCP gas when in combination with a buffer solution. It produces the maximum effectiveness in the air-tight application (Sisler et al., 2006). Aqueous formulations of 1-MCP, which are flexible to apply as pre-harvest spray solutions in the field or as dip or spray treatments during postharvest handling, have been developed in recent years (Manganaris et al., 2007; Choi et al., 2008; Villalobos-Acuna et al., 2010). Seglie et al., (2011a) also investigated a new application method of 1-MCP which is encapsulated in β-cyclodextrins cross-linking with nanosponges. They found that this method is more efficient in releasing the active compound 1-MCP compared to the conventional cyclodextrin complexation. New types of user-friendly 1-MCP products such as SmartTabs™ and EthylBloc™ Sachet are also currently available (Valero et al., 2016).

***Mode of action*:** A ligand substitution model was proposed by Sisler and Serek (1997) to explain the binding action of ethylene to the receptor with the ligands of unknown structure. According to them, ethylene binds to the metal of the receptor by withdrawing electrons. Once the ligand substitution process takes place, ethylene is removed from the receptor and an active receptor complex is formed. The ethylene removal and active receptor formation process is necessary to transmit the signal for the ethylene response. The same binding process occurs in the case of ethylene antagonist 1-MCP. However, 1-MCP binds permanently to the metal cofactor leading to failure of the active receptor formation and the ethylene signal response (Sisler and Serek, 1997).

Pirrung et al. (2008) proposed a new model (Fig. 2.6) for the mode of action of 1-MCP. In this model, 1-MCP binds to the copper(I) cofactor of the ethylene receptor. This binding initiates the ring-opening reaction of 1-MCP resulting in a copper carbenoid complex (Step 1 and 2, Fig. 2.6). The carbenoid complex is highly reactive and covalently bonds with the protein matrix within the ethylene receptor site (Step 3, Fig. 2.6) leaving the receptor inactive. Inactivation of the ethylene receptor will block the ethylene signal response and impede the action of ethylene in plant cells.

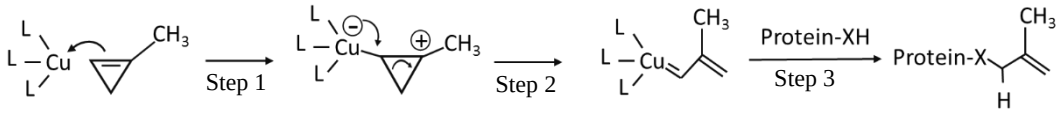
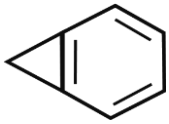


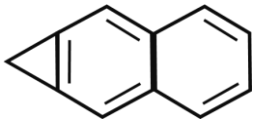
Fig. 2. 6 The proposed ethylene receptor binding mechanism of 1-methylcyclopropene (1-MCP) (Pirrung et al., 2008)

2.2.5.2 1*H*- cyclopropabenzene (BC) and 1*H*-naphtho[*b*]cyclopropane (NC)

Recently, Singh et al. (2018) have patented two new ethylene antagonists 1*H*-cyclopropabenzene (BC) and 1*H*-naphtho[*b*]cyclopropane (NC) to prevent ethylene action in plants (Patent no. WO 2018/049465 AI). They observed that fumigation with BC and NC can inhibit the ethylene action in climacteric fruits. BC and NC belong to a class of compound known as the cycloproparenes which are formed by the fusion of cyclopropene to an aromatic ring (Fig. 2.7) (Halton, 1973). It is proposed that BC and NC react with ethylene receptors in a similar manner to 1-MCP due to the presence of a masked 'cyclopropene' functional group. The ring-opening reactions of cycloproparenes with silver(I) salts are identical to the ones for 1-MCP described by Pirrung et al., (2008) in Fig. 2.6. The proposed mode of action of BC is described in Fig. 2.8. BC binds to the copper(I) cofactor of the ethylene receptor (Step 1, Fig. 2.8), triggering a ring-opening reaction (Step 2, Fig. 2.8) and reacts with the protein matrix in the receptor to form a covalent bond (Step 3, Fig. 2.8). This irreversible binding of BC to the ethylene receptor inactivates it and blocks signal transduction. The proposed mode of action of NC is identical to BC and is shown in Fig. 2.9. BC is a liquid and NC is a solid at room temperature and are stable for long periods which allows easy handling and application (Singh et al., 2018). Investigation on different formulations of these ethylene antagonists, which could be applied either as pre- or postharvest solutions in both open field or airtight storage/shipping conditions, could be of great advantage for the fruit industry.



1*H*- cyclopropabenzene (BC)



1*H*-naphtho[*b*]cyclopropane (NC)

Fig. 2. 7 The chemical structures of 1*H*-benzocyclopropene (BC) and 1*H*-naphtho[*b*]cyclopropane (NC).

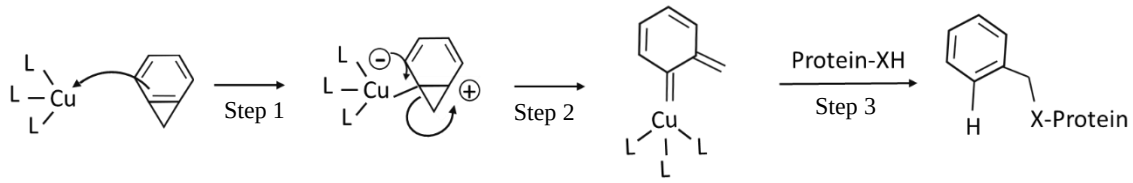


Fig. 2. 8 The proposed ethylene receptor binding mechanism of BC.

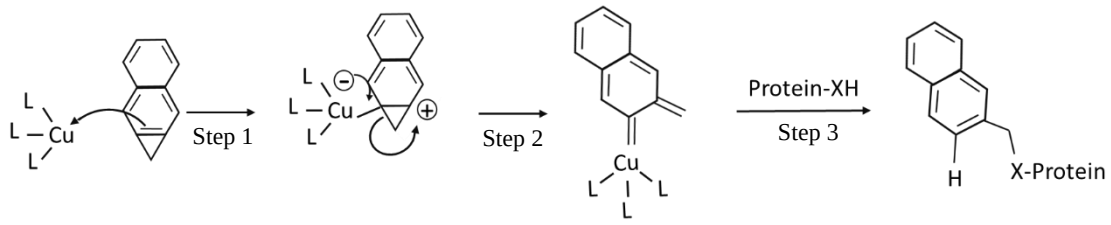


Fig. 2. 9 The proposed ethylene receptor binding mechanism of NC.

2.3 Physio-chemical changes during fruit ripening

Ripening is an irreversible process involving a series of physical and biochemical changes such as the increased activity of softening enzymes, development of fruit colour, biosynthesis of flavour and aroma compounds. Once ripening has started, the fruit initiates senescence leading to shrivelling, complete breakdown and death of fruit tissues (Crisosto and Day, 2012). From the perspective of growers, wholesalers and retailers, ripening needs to be hastened or postponed according to the market demand to preserve fruit quality. On the other hand, the consumers, the ultimate destination of production, demand a fully-ripe fruit with exceptional appearance and highest

nutritional quality. Understanding the physiology of fruit ripening will facilitate us in the regulation of the marketable life and the fruit visual and nutritional qualities using the advanced postharvest management technologies (Khan et al., 2018).

2.3.1 Respiration

Respiration is a metabolic process in which carbohydrate is oxidised into carbon dioxide and water, releasing the required energy for the development of plant parts in the form of ATP. The rate of respiration can be conveyed as the depletion rate of O_2 and/or production rate of CO_2 (Fonseca et al., 2002; Taiz and Zeiger, 2006). After harvest, fruit continues respiration to supply the ATP required for the biochemical processes including ripening and senescence.

Based on the respiration pattern during ripening, fruits are categorised into two groups: (1) climacteric fruit which present a climacteric rise in respiration and ethylene just before or after the onset of ripening and (2) non-climacteric fruit, in which the climacteric respiratory peak is absent (Lelievre et al., 1997). Stone fruits are generally considered as climacteric fruit (Crisosto and Day, 2012), however, there are certain type of plums such as 'Gulfruby' and 'Beauty' which exhibit suppressed-climacteric respiration pattern with almost half of the CO_2 production of climacteric ones like 'Shiro' and 'Rubyred' (Abdi et al., 1997 and 1998).

The postharvest respiration rate of fruit is highly influenced by temperature, oxygen and carbon dioxide concentrations of the storage environment. The respiration rate increases with the rise in temperature till it reaches a plateau, after that it declines even at higher temperatures (Taiz and Zeiger, 2006). Controlling the temperature and modification of the storage atmosphere are common practices to slow down the respiration rates of fruit during postharvest handling practices (Fonseca et al., 2002). Low-temperature storage at 1°C reduced the respiration rate and extended the storage life of 'Laetitia' plums for 20 days compared to the plums stored at 23 °C (Argenta et al., 2003). 'Friar' plums stored at 0 and 2 °C suppressed and delayed the respiration peaks onset 36.9 and 34.4 mg kg^{-1} h^{-1}, respectively for 2-3 weeks than those stored at 5, 15 and 20 °C (Wang et al., 2016). Cantin et al. (2008) reported that maintaining high carbon dioxide and low oxygen levels with different MAP box liners resulted in slower respiration rate preserving fruit quality and improving market life of 'Friar' plums up

to 45 days under cold storage (0 °C). Similar findings were also reported in 'Blackamber', 'Larry Ann', 'Golden Globe' and 'Songold' plums (Diaz-Mula et al., 2011).

2.3.2 Weight loss

Physiological weight loss is one of the important visual quality indexes, which leads to fruit shrivelling and subsequent loss of market value. Weight loss can cause a substantial economic loss especially in the fruit sold by weight (Vigneault et al., 2009). Weight loss is mainly due to the cumulative loss of water content from the fruit (Crisosto and Valero, 2008). It is related to respiration and transpiration in which water diffuses through stomata, lenticels and other openings of the fruit surface (Lara et al., 2014). In some fruit like peaches and nectarines, the loss of approximately 5-8% water content (based on weight at harvest) is sufficient to cause shrivelling (Crisosto and Day, 2012).

The fruit surface has a natural barrier to water loss, called the cuticle. The main function of the cuticle during postharvest fruit maturation is to limit water loss and to protect the fruit from biotic attacks and abiotic stresses (Kolattukudy, 1984; Belge et al., 2014). The barrier function of the cuticle is highly dependent on temperature and relative humidity of the surrounding atmosphere (Lara et al., 2014).

To assure the best postharvest quality of fruit without shrivelling, it is essential to maintain low temperature and high relative humidity throughout the postharvest handling chain (Crisosto and Day, 2012; Lara et al., 2014). Additionally, postharvest treatments such as waxing, coating (Jeong et al., 2003; Shiekh et al., 2013) and modified atmosphere packaging (MAP) practices (Valero and Serrano, 2010b) are the common approaches to reduce water loss, thereby to extend postharvest storage life and to maintain the quality of the fruit. Physiological weight loss of 'Jewel' strawberry was reduced up to 50% when the fruit are stored at 0.5 or 10 °C and 85 or 95 % relative humidity (Shin et al., 2007). Application of 1 or 3 % alginate edible coating before storage significantly reduced weight loss of 'Blackamber', 'Larry Ann', 'Golden Globe' and 'Songold' plums stored for 35 days at 2°C and after 3 days at 20 °C. The weight loss reduction is attributed to the lower transpiration induced by edible coatings (Valero et al., 2013). Coating with 1 % chitosan in combination with lower storage

temperature (0-1 °C) and high relative humidity (90±5 %) resulted in significant weight loss reduction in 'Stanley' and 'Giant' plums stored for 40 days (Bal, 2013). Application of MAP in combination with cold storage at 0±1 °C, 90±5 % RH reduced ≈ 92 % weight loss of 'Tegan Blue' plum as compared to control, regardless of 1 µL L^{-1} 1-MCP treatment. MAP increases the water vapour accumulation and maintains high relative humidity inside the package resulting in a low rate of water loss from the fruit (Khan and Singh, 2008).

2.3.3 Firmness

Firmness is one of the most important fruit maturity indices at harvest (Crisosto, 1994) and more importantly, it is one major quality criteria for postharvest handlers and consumer acceptance of stone fruit (Crisosto, 2001; Zhang et al., 2010). In plum, peach and nectarine, fruit firmness decreases very rapidly during ripening. Reduction in fruit firmness substantially limits postharvest storage life and quality of fruit (Khan and Singh, 2008; Khan et al., 2009). Fruit firmness at commercial harvest ranges between 27.5 - 38.6 newtons (N) in plum (Khan and Singh, 2007 b), 53.7-76.4 N in nectarine (Rath and Prentice, 2004) and 42-51 N in peach (Lleo et al., 2009) depending on cultivar, fruit size and harvest date. The fruit that are ready to be consumed hold the firmness in the range of 9 to 13 N in plums (Puerta-Gomez and Cisneros-Zevallos, 2011) and 12.8-18.0 in peaches and nectarines (Delgado et al., 2013). The fruit firmness is 4.5 N or less at full ripe and overripe stages (Puerta-Gomez and Cisneros-Zevallos, 2011).

Fruit softening is primarily resulting from the enzymatic breakdown of cell wall and degradation of its components: cellulose, pectins and hemicelluloses (Rose and Bennett, 1999; Ramina et al., 2008). The major enzymes that are mainly responsible for fruit softening are polygalacturonase and β-(1,4)-glucanase and their activities increase with the commence of ripening (Brady, 1993; Ramina et al., 2008). The increased activities of cell wall degradation enzymes, endo- and exo-polygalacturonase, pectin esterase and endo-1,4-β-D-glucanase, during fruit ripening, were reported in Tegan Blue' plum (Khan and Singh, 2008), in nectarine (Zhou et al., 2000) and peach (Brummell et al., 2004). Fruit softening is partially influenced by ethylene in some fruit like melon, whilst it is totally ethylene dependent in certain fruit like tomato and peach (Hayama, 2006a and b; Pech et al., 2012).

Regulating the postharvest ethylene production can slow down fruit softening and prolong the storage or shelf life of stone fruit such as peach, nectarine and plum. Liguori et al., (2004) demonstrated that 'Almog' and 'Oded' peaches, and 'April Glow' nectarine treated with different concentrations of 1-MCP (0.5, 1, 5 and 20 µL L^{-1}) at 0 and 20 °C for 5, 10 and 20 h were firmer than the control fruit. They concluded that the effectiveness of 1-MCP in slowing down the softening is dependent on concentration and exposure time of the treatment. Similarly, 'Tegan Blue' plum treated with different concentrations of 1-MCP (0, 0.5, 1.0 and 2.0 µL L^{-1}) showed a significant reduction in the activities of enzymes responsible for fruit softening in both skin and pulp. The reduction was more pronounced in higher concentrations of 1-MCP (Khan and Singh, 2007a). The 'Akatsuki' peach treated with 1 µL L^{-1} of 1-MCP for 16 h in combination with 150 mg L^{-1} of AVG at 25 °C significantly maintained fruit firmness throughout the ripening period up to 9 d (Hayama et al., 2008).

2.3.4 Soluble solids content (SSC), titratable acidity (TA) and SSC: TA

In stone fruits, soluble solids content (SSC) and titratable acidity (TA) are one of the major organoleptic criteria for consumer acceptance (Crisosto and Day, 2012). TA is also an important identification of optimal harvest maturity (Khan et al., 2018). The major components of soluble solids content in ripe fruit are sugars (more than 60%) and organic acids, along with other minor constituents like phenols, soluble pectins and minerals (Kader, 2008, Ramina et al., 2008). Although the correlation between sweetness and SSC content is low (Kader, 2008), SSC is commonly expressed as the sweetness of fruit because of its quick and easy accessibility (Beckles, 2012). In general, the soluble solids content of fruit increase during ripening (Khan et al., 2018).

The titratable acidity in stone fruits is normally represented by malic acid content and it tends to decrease during fruit ripening (Khan et al., 2018). The fruit with lower TA value (less than 1%) during ripening can attain high consumer acceptance (Crisosto et al., 2004; Delgado et al., 2013). According to Valero and Serrano (2010a), the ratio between soluble solids content and titratable acidity (SSC:TA), rather than either of them alone, is a better indicator of consumer acceptance. Peaches with high SSC content (minimum of 11%) and low TA (less than 0.7%) hold high consumer acceptance (Crisosto and Day, 2012). Similarly, plums with SSC content of ≥12% combined with lower TA contents attain more consumer preference according to the

in-store evaluation by 100 consumers in California (Crisosto et al., 2004). Consumer acceptability of seven nectarine and plum cultivars from California were judged by 120 consumers based on five quality attributes including soluble solid concentration and titratable acidity. The evaluation revealed that sweetness is the main sensory attribute for consumer perception (Delgado et al., 2013). However, the consumer acceptance value for SSC, TA and SSC:TA ratio may vary depending on fruit cultivars and places of consumption (Crisosto and Kader, 2000).

2.3.5 Individual sugars and organic acids

Stone fruit are a rich source of sugars and organic acids. The composition of sugars and organic acids plays a remarkable role in overall flavour of stone fruit improving consumer acceptance (Ramina et al., 2008; Khan et al., 2018). During fruit maturation and ripening, the stored carbohydrates are hydrolysed into sugars and the sugar concentrations increase with the commencement of ripening (Valero and Serrano, 2010a). In general, the predominant sugars during stone fruit ripening are sucrose, glucose, fructose (Ramina et al., 2008; Valero and Serrano, 2013; Khan et al., 2018) and sorbitol (sugar-alcohol) (Valero and Serrano, 2010a). However, their proportion and concentration during ripening may vary depending on fruit cultivar (Brady, 1993; Valero and Serrano, 2013; Dugalic et al., 2014). Sucrose tends to be the major sugar in peaches and nectarines and its concentration is the highest during ripening (Ramina et al., 2008; Thakur and Singh, 2012), whilst in plum varieties fructose is the predominant sugar (Singh and Singh, 2008).

Organic acids are more abundant during early stages of fruit maturation and the concentrations gradually decrease with the commencement of ripening (Valero and Serrano, 2013). The predominant organic acid during ripening of peach, nectarine and plum is malic acid which comprises 75-93% of the total acids and its concentration declines over the ripening processes (Wills et al., 1983; Valero and Serrano, 2010a). Other organic acids, such as citric, succinic, fumaric, quinic, oxalic and tartaric acids are also found during stone fruit ripening but at negligible concentrations (Ramina et al., 2008, Singh et al., 2009; Valero and Serrano, 2010a).

2.3.6 Bioactive compounds: vitamins, phenols and anthocyanin, and antioxidant activity

Stone fruit, similar to other horticultural crops, are good sources of bioactive compounds, such as phenols and vitamins, with abundant health-benefiting properties. They are the secondary plant metabolites and contribute to the antioxidant capacity and are responsible for the colour, taste and flavour of the fruit (Cantin et al., 2009; Yalcin and Capar, 2017). The abundance of antioxidants in stone fruit has lead researchers to investigate market opportunity in their potential as ingredients in pharmaceutical industries, functional food market and cosmetic industries (Puerta-Gomez and Cisneros-Zevallos, 2011).

Phenols are the compounds which contain an aromatic ring with one or more hydroxyl group attached. They are ubiquitous in the plant world and are involved in pigmentation, growth, resistance to pathogens and as antioxidant agents in plants. The major phenolic compounds in fruit are phenolic acids, hydroxycinnamic acids, stilbenes and flavonoids. The total phenolic concentration in plums increases with the advancement of fruit maturation (Valero and Serrano, 2010a). Anthocyanins are phenolic compounds, categorised under flavonoids, responsible for the colour changes during fruit ripening. These water-soluble pigments are responsible for the purple and red colour of the fruits. The major anthocyanin in red-purple plums is cyanidin 3-glucoside with the concentrations of 100-800 and 2-100 mg per 100 g in skin and flesh, respectively (Fanning et al., 2014; Sahamishirazi et al., 2017). Ascorbic acid, also known as vitamin C, is the important water-soluble vitamin in human nutrition. It is also an effective antioxidant in scavenging the reactive oxygen species (ROS) and in inhibiting lipid peroxidation. In general, the ascorbic acid concentration was higher in immature fruit than in the mature ones. However, the ascorbic acid content in fruit may vary depending on cultivars and several pre-harvest and postharvest factors (Lee and Kader, 2002). In general, levels of ascorbic acid in peaches and nectarines were comparatively higher compared to plums, however, there were exceptions in cultivars such as ‘Redhaven’ peach and ‘Wilson’ plum which hold similar ascorbic acid concentration (Wills et al., 1983; Gil et al., 2002). The environmental factors, such as temperature, light and altitude and cultural practices such as fertilization and irrigation, also influence the ascorbic acid content of fruit (Lester, 2006). Mulching with 5-8 cm

thick layer of wheat-straw to planting beds increased 1.5 fold ascorbic acid content in 'Bounty' strawberries compared to mulching with 0.06 mm thick black polyethylene plastic sheet (Moor et al., 2005). The postharvest treatments with 1, 2, 3 or 4 mM of either putrescine or salicylic acid significantly reduced the degradation of ascorbic acid content in 'Santa Rosa' plum stored at 4 °C for 25 days (Davarynejad et al., 2015).

Phenolic compounds are the major contributing bioactive compounds for the antioxidant capacity of peach, nectarine and plum, however, the ascorbic acid and carotenoids also play a role in the antioxidant capacity (Gil et al., 2002; Cantin et al., 2009; Cosmulescu et al., 2015; Yalcin and Capar, 2017). It was reported that carotenoids are the main responsible for total antioxidant capacity in yellow plum cultivar, peach and nectarine while anthocyanin in red and purple colour plum (Crisosto and Valero, 2008). In general, plums contain more phenolic compounds, thus more antioxidant activity, compared to peaches and nectarines (Gil et al., 2002). However, the composition and level of bioactive compounds in stone fruit can vary depending on cultivar, harvest maturity, an environmental condition during postharvest handling and other several factors (Cantin et al., 2009; Cosmulescu et al., 2015; Yalcin and Capar, 2017). Gil et al. (2002) evaluated total phenolic, ascorbic acid and antioxidant capacity of 25 different cultivars of peach, nectarine and plum in California. They found that the ascorbic acid contents were in the range of 3-14 mg per 100 g of fresh weight, while the total phenolic contents ranged from 14 to 111 mg per 100 g fresh weight. The antioxidant capacity was highly correlated to the phenolic contents in all the tested cultivars.

2.4 Regulation of ethylene to manage fruit ripening, quality and storage life

Regulation of ethylene at any stage, from biosynthesis to the ethylene receptor, can facilitate managing storage life, aesthetic and nutritional quality of fruit (Nath et al., 2006). In plums, inhibiting or promoting ethylene production is a postharvest treatment used to delay fruit ripening or to increase the availability of fruit in the market, respectively (Manganaris et al., 2008; Pan et al., 2016).

2.4.1 Improving ripening and fruit quality

Exogenous application of ethylene is a well-known postharvest practises in fruit to promote ripening according to market demand. Banana fruit treated with ethylene (any of 0.01, 0.1 and 1 mL L^{-1}) for 24 h at 18 °C showed a climacteric rise in respiration, increase soluble solids content and colour development. Other compounds can also be used, for instance, fruit treated with 1 mL L^{-1} of acetylene exhibited the similar colour score and soluble contents as of the fruit treated with ethylene (Thompson and Seymour, 1982). In mango, treatment with 0.01, 0.1 and 1 mL L^{-1} of ethylene or 1 mL L^{-1} of acetylene for 24 h at 25 °C induced ripening and the related processes such as textural changes, acidity reduction and colour development (Medlicott et al., 1987). In 'Ataulfo' mango stored at 13±1 °C for 4 d, 100 µL L^{-1} ethylene treatment for 12 h enhanced the ripening by stimulating activity of ACC synthase and ACC oxidase, the enzymes responsible for ethylene biosynthesis (Montalvo et al., 2007). The ethylene releasing compound, ethephon (commercially available as Ethrel®), has also been reported as a ripening promoter in different kinds of fruits such as apple (Wang and Dilley, 2001), mango (Nair and Singh, 2003), pear (Dhillon and Mahajan, 2011) and tomato (Dhall and Singh, 2013). Ethylene also promotes colour development in non-climacteric fruit though chlorophyll degradation and/or biosynthesis of pigments. Application of 5000 µL L^{-1} propylene, an ethylene agonist, to green strawberries induces colour development (Perkins-Veazie et al., 1996). In 'Cabernet Sauvignon' grape, application of 140±18 ppm of ethylene for 24 h induces skin colour development by stimulating long-term expression of the genes related to anthocyanin biosynthesis (El-Kereamy et al., 2003). In 'Aoshima Satsuma' mandarin, application of 1000 µL L^{-1} ethylene for 24 h at 20 °C promotes carotenoid accumulation in flavedo by enhancing the expression of the genes responsible for carotenoid biosynthesis (Matsumoto et al., 2009). It was reported that application of 500 µL L^{-1} propylene, the agonist of ethylene, at the flow rate of 2 L min^{-1} induced sucrose accumulation while it decreased sorbitol accumulation in climacteric plum 'Santa Rosa' and non-climacteric plum 'Sweet Miriam' (Farcuh et al., 2018). Several other plant hormones which stimulate ethylene biosynthesis can also be applied as fruit ripening promoters, such as auxin in mango (Zaharah et al., 2012), methyl jasmonate in plum (Khan and Singh, 2007b), brassinosteriods in different types of fruit (Aghdam et al., 2016a).

2.4.2 Delaying fruit ripening and maintaining quality

To control the effect of ethylene during postharvest handling, inhibition can be done either at the biosynthesis level or at the receptor level. It is impossible to control the effect of ethylene on fruit during postharvest handling by regulating the ACC biosynthesis pathway or ACO related enzyme activities or gene expressions. There are several sources of external ethylene during fruit storage and transportation which cause the same effects. These sources include combustion engines, overripen/ rotten fruits and vegetables along the postharvest supply chain (Gibson et al., 2000; Wills et al., 2014). In this regard, different compounds have been developed to inhibit the action of ethylene in plant parts by blocking the receptors (Sisler and Serek, 2003).

2.4.2.1 Inhibition of ethylene biosynthesis

Ethylene biosynthesis can be minimised by suppression of the enzymes, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase, involved in its biosynthesis pathway (Yang and Hoffman, 1984; Kende, 1993). Aminoethoxyvinyl glycine (AVG) is commonly used to inhibit ethylene biosynthesis by reducing the activity of ACC synthase enzyme (Yang and Hoffman, 1984). Preharvest or postharvest application of AVG can reduce ethylene production and downregulate other ethylene-responsive postharvest parameters such as fruit softening in plum, peach and apricot (Belding and Lokaj, 2002; Palou and Crisosto, 2002; Hayama et al., 2008; Kucuker et al., 2015; Tareen et al., 2017). Inorganic ions such as Co^{2+} and Ni^{2+} are the effective ethylene biosynthesis inhibitors by inhibiting the activity of ACC oxidase (Yang and Hoffman, 1984). Polyamines are also an alternative approach to inhibit ethylene biosynthesis by competing for *S*-adenosylmethionine (SAM), the precursor of ethylene biosynthesis pathway (Serrano et al., 2016). Pre- or postharvest application of putrescine (1,4-diaminobutane) can delay fruit ripening and the related processes such as fruit softening, sugars and ascorbic acid accumulation by reducing ethylene production in Japanese plum (Singh and Khan, 2010). Salicylic acid can also reduce ethylene-induced fruit ripening of banana, kiwi and mango through reduction of ACC and ACO enzymes activity and their gene expression (Aghdam et al., 2016b).

2.4.2.2 Inhibition of ethylene action at the receptor level

Inhibiting the action of ethylene is alternative postharvest management. Different compounds are used to inhibit ethylene action by blocking the ethylene receptors. These ethylene antagonists are commonly cyclopropenes, with 1-MCP being the most famous. Sisler et al. (2003) examined a series of substitute cyclopropenes for their blocking efficiency of ethylene action in banana and found that 1-MCP application protects the ethylene action for 12 d with minimum concentration at 0.7 nL L^{-1} for 24 h while 1-decylcyclopropene resulted to be the longest ethylene protection compound (for 34 d). Feng et al. (2004) also reported that application of 1-ethylcyclopropene and 1-propylcyclopropene for 24 h at 22 °C delayed ripening of avocado and tomato by inhibiting the action of ethylene. Sisler et al. (2009) investigated the effects of five different dialkylamine compounds and one derivative of 1-methylcyclopropene to counteract the action of ethylene in banana. All of them showed a similar inhibitory effect on ethylene response up to 32-34 d. Xu et al. (2014) reported that application of 1-pentylcyclopropene (1.2 µL L^{-1}) for 20 h at 20±2 °C can inhibit the action of ethylene in 'Brazil' banana by suppressing the gene expression of ethylene receptors and by reducing the enzyme activities related to ethylene biosynthesis. Similar results on inhibition of ethylene action in 'Shenyang' tomato by application of 1-octylcyclopropene (1.2 µL L^{-1}) for 20 h at 20±2 °C was reported by Xu et al., (2016). Although many compounds are proved to be effective in inhibiting the action of ethylene, it is still only in laboratory conditions. Presently, 1-methylcyclopropene (1-MCP) is the most commonly used commercial ethylene antagonist (Valero et al., 2016). The benefits of the 1-MCP application in blocking the ethylene action to delay ripening and the related biochemical processes on different types of fruit have been extensively reviewed by Blankenship and Dole, (2003), Watkins (2006 and 2015) and Valero et al. (2016).

In general, ethylene biosynthesis and action inhibitors are applied along with storage management practices such as MAP and cold storage. The synergistic effect of ethylene antagonists and MAP in retaining fruit firmness, prolonging storage life and preserving the postharvest quality of fruit has been demonstrated by Oz (2011) and Ozkaya et al. (2016). Khan and Singh (2009) reported that fumigation of ethylene antagonist, 1-MCP (1 and 2 µL L^{-1}), prolonged the storage life of 'Tegan Blue' plum

by retaining fruit firmness and reducing ethylene production under cold storage (0±1°C).

2.4.2.3 Removing ethylene from the storage environment

During postharvest handling of the fruit, there are several external sources of ethylene like combustion engines and rotten fruit and vegetables (Gibson et al., 2000). The presence of a small amount of ethylene in the storage atmosphere can accelerate the plant internal biosynthesis process. Both endogenous and exogenous ethylene show the same effect on the fruit by accelerating ripening and senescence processes during storage. Ethylene can be removed from the storage and handling environment by using oxidizing agents such as potassium permanganate ($KMnO_4$) and ozone (O_3) (Keller et al., 2013, Hu et al., 2019). The application of $KMnO_4$-based ethylene scrubbers is one of the most effective postharvest tools to extend the storage life of fruit and vegetables. Different materials such as vermiculite, activated alumina, zeolite, silica gel, activated carbon and clays are used as supportive material to improve oxidization process of $KMnO_4$ (Alvarez-Hernandez et al., 2018). Photocatalytic oxidation with nanoparticles of titanium dioxide (TiO_2) was also reported as an effective ethylene removal method intomato (de Chiara et al., 2015).

2.5 Regulation of storage environment to maintain fruit quality and storage life

Although many postharvest strategies have been developed and are being practised in commercial storage of horticultural crops (Keller et al., 2013), modifying the storage atmosphere and temperature are still the fundamental practices to preserve the quality, to extend the storage life and to avoid the accumulation of exogenous ethylene. Managing the storage atmosphere and temperature can also indirectly inhibit ethylene biosynthesis and action by controlling the biochemical processes of the produce.

2.5.1 Cold storage

The storage temperature is one of the important keys to the success of postharvest technologies as it slows down the metabolic process of the produce (Hoehn et al., 2009). It is also a crucial factor to maintain visual and nutritional quality during

postharvest handling. The physiological and biochemical mechanisms which trigger the quality deterioration of the fruit during storage are generally controlled by temperature (Valero and Serrano, 2010a). The higher the temperature, the faster the respiration rate and the greater chance of creating anaerobic conditions which could lead to the production of ethanol and acetaldehyde. The accumulation of these anaerobic metabolites induces the development of off-flavour and disorders during storage of fruit (Pesis, 2005). Cold storage has been practised as a fundamental postharvest approach to reduce fruit softening, to delay fruit ripening, shrivelling and to prevent the development of postharvest diseases and physiological disorders (Crisosto et al., 1995). Integrated approaches, i.e. cold storage along with other postharvest technologies such as CA, MAP and ethylene biosynthesis inhibitors, can synergistically improve the effectiveness in retarding ripening processes, extending storage life and maintaining the fruit quality during storage and transportation (Kader, 1993; Menniti et al., 2004; Khan et al., 2009). MAP in combination with cold storage reduces weight losses and maintains eating quality attributes like total soluble solids, titratable acidity, and flesh firmness of fruit without any detrimental effects in peach (Akbudak and Eris, 2004). Despite the benefits in extending storage life and reducing postharvest losses, prolong cold storage (below 10°C) can induce chilling injury, one of the main factors for postharvest quality deterioration in stone fruit (Crisosto and Day, 2012). Therefore, it is important to know the optimum temperature for cold storage of each fruit. According to Crisosto and Day (2012), the optimum cold storage temperature for stone fruit is -1°C to 0°C.

2.5.2 Modified atmosphere packaging (MAP)

MAP is a well-recognised postharvest technology to facilitate storage life extension, quality assurance and safety of fresh and fresh-cut horticultural commodities. MAP is a technology where the atmosphere inside packaging has elevated carbon dioxide and reduced oxygen levels through respiration of the stored produce (Kader et al., 1989). Kader and Watkins (2000) have defined that MAP is maintaining the required optimum atmosphere to extend the marketable life either by respiration of the produce (passive MAP) or by introducing the desired gas mixture into the package (active MAP). In passive MAP, according to the authors, respiration of the commodity depletes oxygen and builds up carbon dioxide inside the package atmosphere, whilst

the packing films are responsible for the diffusion of the gases through the package. Thus, the interaction between permeability properties of packing material and the respiration rate of produce is important to maintain the equilibrium gas composition (Hoehn et al., 2009).

The major gases involved in passive MAP are:

(a) Oxygen: Being living organisms, fresh fruit require a sufficient amount of oxygen mainly for their respiratory process and other metabolic reactions. From the perspective of postharvest technology, reduction of oxygen (below 10 kPa) in the surrounding atmosphere of the fruit can slow down the metabolic processes of the fruit leading to delayed ripening process and extended storage life (Brandenburg and Zagory, 2009). Nonetheless, the concentration below 1-2 kPa can create anoxic condition triggering quality deterioration such as the development of off-flavours (Valero and Serrano, 2010b). In addition, low level O_2 concentrations along with poor temperature management may also lead to the development of microbial spoilage inside the package which is one of the causes of quality deterioration (Brandenburg and Zagory, 2009).

(b) Carbon dioxide: Carbon dioxide, one of the respiratory products, is also another crucial gas which controls the success of MAP. Elevating the level of CO_2 (>10 kPa) inside the package enables the suppression of respiration rate of the produce to a certain extent which leads to prolonged storage life (Brandenburg and Zagory, 2009). It can suppress the development of pathogenic organisms which is caused by the decreased O_2 concentration during storage. In addition, carbon dioxide can suppress the ethylene biosynthesis and thus, subsequently diminish the adverse effects of ethylene (Sisler, 2006; Brandenburg and Zagory, 2009). However, the CO_2 concentration higher than the critical value will lead to the development of physiological disorders (Valero and Serrano, 2010b) during storage triggering the loss of visual and nutritional quality.

The ideal concentration of CO_2 and O_2 for MAP may vary depending on the cultivars. An et al. (2007) reported that the MAP film which provided the equilibrium concentration of 5 kPa CO_2 and 4 kPa O_2 was found to be the best quality of 'Chaoyang' honey peach. To obtain the optimum gas composition for the success of

MAP in extending the storage life and quality maintenance, the packing materials should be designed according to the respiration rate of individual produce (Kader and Watkins, 2000; Valero and Serrano, 2010b). Different kinds of commercial MAP bags, such as Lifespan®, which are specifically designed to use during storage and transportation for each type of fresh fruit are available in the market.

In addition to the permeability of CO_2 and O_2 gas, the packing materials used in MAP limit the diffusion of water vapour through the package. This allows generating water vapour pressure inside the package reducing evaporation and thereby reducing the weight loss, which is one of the important postharvest quality criteria for those traded based on a weight basis (Valero and Serrano, 2010b).

However, Crisosto et al., (2009) reported that the use of MAP technologies in peaches and nectarines has limited benefits on maintaining postharvest quality as it creates condensation, increases the incidence of decay and development of flesh mealiness and browning during transportation. In contrast, unlike in peach and nectarine, the use of MAP to extend storage life and assure postharvest quality of plum has been reported to be successful in 'Angeleno' plum (Peano et al., 2017), 'Friar' plum (Cantin, Crisoto and Day, 2008) and 'Tegan Blue' plum (Khan and Singh, 2008). The differences in surface morphological structures of stone fruits, especially the presence of visible epicuticular waxes in plum, may presumably be the reason for these variations in the effectiveness of MAP. Martin and Rose, (2014) have reported that fruit cuticle limits the permeability of water into or out of fruit preventing transpiration and, thus water loss. It has also been reported that the activities of ethylene biosynthesis enzymes and softening enzymes in 'Tegan Blue' plum were effectively reduced by MAP in combination with 1-MCP, leading to delayed fruit ripening while maintaining the quality (Khan and Singh, 2008). The positive impacts of MAP can be enhanced in combination with proper postharvest handling procedures, temperature control management and advanced postharvest treatments (Kader et al., 1989). In the present research, the effectiveness of new ethylene antagonist formulations were examined under MAP storage for better quality maintenance and storage life extension of plum and pluot.

2.6 Fruit cuticle: a natural barrier to postharvest treatments

2.6.1 Composition of fruit cuticle

Fruit cuticle, similarly to cuticles of the other plant parts, is a hydrophobic lipid layer existing as a complex mixture of cutin and cuticular waxes on the outer surface of the fruit (Martin and Rose, 2014; Lara et al., 2015). The cuticles are mainly composed of polyester polymer rich in fatty acids and are embedded in the wax layer (Lara et al., 2014). The waxes layers can be found as epicuticular wax which can easily be seen on the plant surface and intracuticular wax which are embedded with the cuticle (Koch and Ensikat, 2008). Additionally, there can be significant amounts of polysaccharides and flavonoids in some of the fruit cuticles (Lara et al., 2015).

2.6.2 Properties of fruit cuticle

The main physiological functions of fruit cuticle are prevention of water loss by minimising transpiration and reduction of essential solutes diffusion. Protection against water loss is mainly due to the intracuticular waxes (Koch and Ensikat, 2008). In addition, the epicuticular waxes layer of fruit cuticle protects the fruit surfaces from pathogenic attack by minimising adhesion of microbes due to their hydrophobic self-cleaning property (Koch and Ensikat, 2008; Lara et al., 2014). The epicuticular waxes are sometimes easily visible as a white or bluish colouration substances on the fruit surface in some fruit like plum and grape (Koch and Ensikat, 2008).

Martin and Rose (2014) reviewed that fruit cuticles influence the postharvest quality and storage life by playing a key role in preventing desiccation and biotic invasions. Fruit cuticle also has a significant role in physiological weight loss representing as a barrier for water loss by respiration and transpiration during postharvest storage. However, the water permeability of cuticle may vary depending upon cuticle thickness and waxes composition of the individual type of fruit (Lara et al., 2014). Since water loss is one of the important determinants in fruit softening, fruits which have well-developed and thick cuticle structure such as tomato and blueberry retain firmness for longer (Saladie et al., 2007; Paniagua et al., 2013). Understanding the influence of fruit cuticular waxes on postharvest quality has led to the development of artificial waxing and edible coatings in commercial postharvest storage of fruit (Asrey et al., 2008).

2.6.3 Fruit cuticle: A barrier to postharvest treatments

Despite the beneficial barrier properties, fruit cuticle is a challenging obstacle for the preharvest and postharvest treatments in delivering treatment action to the target fruit cells. The cuticle limits direct contact of the active ingredients and the targeted plant parts resulting in a reduction of treatment efficacy. The undesirable barrier properties of plant cuticle inhibiting the uptakes of foliar nutrient application, pesticides, fungicides, pre and post-harvest treatments have been well-documented (Farag et al., 1992; Roy et al., 1996; Fernandez and Eichert, 2009; Singh and Khan, 2012; Castro et al., 2014; Zhou et al., 2014). Limited research has been undertaken to overcome the hindrance of cuticle against agrochemicals by altering the morphological structure of epicuticular wax using different kinds of adjuvants such as surfactants and alcohols in fruits (Roy et.al., 1996) and leaves (Fernandez and Eichert, 2009) without affecting their beneficial barrier properties (Harker and Ferguson,1991).

2.7 Adjuvants used in postharvest treatments

The term adjuvants is applied to any substances which are added to a formulation or a spray solution in order to enhance the activity of the active ingredient or spray characteristics (Fernandez and Eichert, 2009; Somervaille et al., 2012). The use of adjuvants is essential in agrochemical formulations such as pesticides, herbicides, foliar nutrients, growth regulators, pre- and post-harvest treatments not only to improve physical stability of the active ingredients but to enhance their performance (Singh et al., 1999; Knowles, 2008; MacKinnon et al., 2009; Somervaille et al., 2012; Singh and Khan, 2012; Fagerstrom et al., 2013; Castro et al., 2014) and to overcome barrier properties of the plant cuticle (Farag et al., 1992). The adjuvant cyclodextrin can facilitate the controlled release of active compound allowing to be more functional with the safer mode of application (Morillo, 2006). In this thesis, the non-ionic surfactant ‘Tween® 20’, the co-solvent ‘ethanol’ and the carrier compound ‘β-cyclodextrin’ were used as adjuvants in formulations of ethylene antagonists to improve the solubility and stability of active compounds and to overcome the barrier properties of the fruit cuticle.

2.7.1. Surfactant

2.7.1.1 Properties of surfactant

Surfactants are common adjuvants in agrochemical formulations to facilitate the action of the active ingredient and to alter the surface cuticle structure (Miller and Westra, 1998). According to Stock and Holloway (1993), the possible mechanisms of the surfactant action is that they increase the contact area of deposits, disorganise the epicuticular waxes, solubilise the agrochemicals in deposit area and thus, increase the uptakes of agrochemicals. The surfactants are large molecules with an amphiphilic structure. They possess a nonpolar hydrophobic portion that is attached to a polar or ionic hydrophilic group. The hydrophobic part reacts with the surface cuticle of plant parts while the hydrophilic part helps active ingredients to be more soluble resulting better infiltration of agrochemicals (Stock and Holloway, 1993). Surfactants are categorised into anionic, cationic, non-ionic and amphoteric surfactants based on the nature of the hydrophilic group. The non-ionic surfactants are the most commonly used adjuvants in agrochemical formulations due to their low cost and ease of storage. More recently, amphoteric surfactants which have similar properties to non-ionic surfactants are also being applied to agrochemicals and pharmaceutical formulations (Castro et al., 2014; Dragicevic et al., 2015).

Surfactants are generally applied to enhance the effectiveness of agrochemicals such as herbicides (Basu et al., 2002; Ramsey et al., 2005), fungicides (Fagerstrom et al., 2013), insecticides (Parr and Norman, 1965), fruit thinning chemicals (Dennis, 2000), preharvest (Khan et al., 2007) and postharvest treatments (Singh et al., 1999) by facilitating the capacity of emulsification, dispersion, adhesion and retention of the agrochemicals (Parr and Norman, 1965; Myers, 2006). They also facilitate the reduction of surface tension and enhance surface wetting (Myers, 2006; Fernandez and Eichert, 2009). Singh and Khan (2012) reviewed the application of surfactants or adjuvant in the spray solutions to improve the uptakes of different foliar micronutrients in citrus. One of the main reasons for using surfactants in agrochemical applications is to increase the trans-cuticle transport of treated compounds by weakening the barrier properties of the cuticle (Zhou et al., 2014). It has been observed that octylphenoxy surfactants improve the trans-cuticular penetration of plant growth regulator 2-(1-naphthyl)acetic acid (NAA) by increasing the diffusion coefficient in isolated tomato

fruit cuticle (Knoche and Bukovac, 1993). According to Alexander and Hunsche (2016) surfactants improve the penetration rate of manganese (Mn) foliar fertiliser applications in enzymatically isolated tomato fruit cuticles. Fagerstrom et al., (2013) have reported that surfactants induce the changes in cuticular membrane structure and solubilise intra-cuticular lipids facilitating solute mobility, thereby improving the efficacy of systemic agrochemicals. Some surfactants, e.g. Triton X-100 would give the greatest penetration of the active compound by disrupting the major components of epicuticular wax (Roy et.al., 1996), however, these can lead to greater injuries of the cuticle, reduce its barrier effects which protect from biotic and abiotic invasion to the fruits (Curry, 2008).

Non-ionic surfactants are widely used in agrochemical formulations and their hydrophilic components are generally polymerised glycol ether groups. They are synthesised from alkylphenols, fatty alcohols, fatty acids, fatty amines or fatty acid amides by reacting with ethylene or propylene oxides (Castro et al., 2014). The non-ionic surfactants with low polyethylene glycol content are good spreaders and have low surface tension (Dragicevic et al., 2015). Tween® 20 is the member of polysorbates which are non-ionic surfactants with different fatty acid chains of 20, 40, 60 and 80. In the commercial, polysorbates are available as Tween® series (Kerwin, 2008) and out of which, Tween® 20 is the most frequently applied surfactant in the different field due to its non-toxicity, affordability and chemical stability (Yang, 2008). The structure of Tween® 20 is shown in Fig. 2.10.

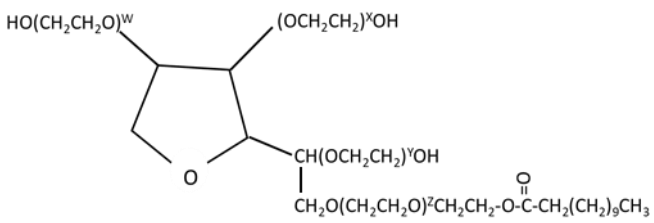


Fig. 2. 10 Chemical structure of Tween® 20: polyoxyethylene sorbitan monolaurate (Source: Kerwin, 2008)

2.7.1.3 Applications of Tween® 20 in postharvest treatments

Tween® 20 is an extensively used surfactant in food (Silva et al., 2012), pharmaceutical (Dragicevic et al., 2015) industries and in the agriculture sector. In the agriculture industry, it has been used for different purposes. Tween® promotes water retention of potting mixtures and increases water use efficiency while increasing plant growth of Peace lily and New Guinea Impatiens flowers when it is used as an adjuvant in irrigation practices (Yang, 2008). Addition of 2500 ppm Tween® during petal distillation of rose flower increased the extracted essential oil content (Baydar & Baydar, 2005). Singh et al. (2000) have examined the efficacy of surfactants on calcium uptake during postharvest MAP storage of mango fruit at 13.5±0.5°C. They revealed that mango fruit treated with calcium chloride solution containing 0.2% of surfactants (including Tween® 20) enhanced the calcium uptake compared to control in both skin and flesh of mango retaining fruit quality and storage life. Khan et al., (2007) included 0.01% Tween® 20 as an surface-active agent in examination of putrescine effect on the biosynthesis of ethylene and softening enzymes in 'Angeleno' plum under Low-temperature storage. Choi et al., (2009) demonstrated that the presence of surfactant (0.2% Tween® 20) can facilitate the retention of aqueous 1-MCP solution (3.7 mmol m^{-3} concentration), which was applied as a postharvest spray to tomato fruits, by improving the solubility of 1-MCP in water. Similarly, Villarreal et al., (2010) applied ethephon (ethylene releasing agent) with 0.02% 'Tween® 20' and 1% ethanol, to improve the solubility and permeability of ethephon, in the investigation of ethylene effect on non-climacteric fruit ripening and enzyme activities. Goren et al., (2011) also used Tween® 20 (0.025%) as a permeability enhancer in preparation of 3-cyclopropyl-1-enyl-propanoic acid sodium salt (CPAS) aqueous solutions to investigate the effectiveness of CPAS on ethylene-related ripening processes of banana. Based on the above examples, it was hypothesised that addition of 'Tween® 20' in formulations of ethylene antagonists will facilitate delivering the antagonist compounds to the targeted ethylene receptors which are located on the ER membrane in the cells of fruit.

2.7.2. Co-solvent: Ethanol

2.7.2.1 Properties of co-solvent

The use of co-solvents is a highly effective technique to enhance the solubility of poorly soluble agrochemicals and sometimes can enhance the biological efficacy of agrochemicals (Knowles, 2008). Alcohols are one of the most commonly used co-solvents with the ability to interact with both hydrophobic and hydrophilic compounds (Klemm, 1998). Ethanol is short-chain alcohol with an amphiphilic molecular structure composed of a polar hydroxyl head group and a non-polar hydrocarbon chain tail (Fig. 2.11). The amphiphilic nature of ethanol allows it to be water-soluble and easily diffuse through lipid bilayers (Dickey and Faller, 2007). Ethanol is one of the most frequently used polar co-solvents due to its low toxicity. It has a strong effect in altering the structural properties of lipid bilayers triggering greater fluidity and permeability (Patra et al., 2006). Due to readily available at affordable cost, ethanol is a promising compound for the production of a variety of value-added chemicals (Sun and Wang, 2014).

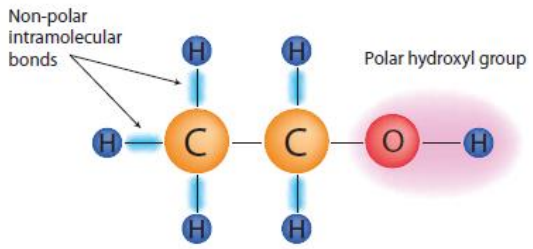


Fig. 2. 11 The structure of ethanol

2.7.2.2 Applications of ethanol

The disruptive effect of ethanol on cell membranes has been well-recognised (Goldstein, 1986) and has been used in pharmaceutical industries to enhance drug delivery (Vemula et al., 2010). In agriculture, ethanol is used to promote the solubility of hydrophobic organic compounds (Otero-Diaz et al., 2017) and to increase the penetration across plant surface, and in biochemical assays as extraction solvent (Sun

et al., 2015). Grichko (2006) demonstrated that ethanol can be used as a solvent in the preparation of spray solutions for poorly water-soluble cyclopropenes to increase their solubility. Farag et al. (1992) observed that the addition of ethanol to ethephon treatments containing tergitol (surfactant) enhances anthocyanin content of cranberry up to 40% as compared to ethephon containing tergitol alone. It was concluded that ethanol increases the diffusion, surface binding and partitioning of ethephon across the cranberry fruit cuticle which enhances the performance of ethephon in increasing anthocyanin accumulation.

2.7.2.3 Applications of ethanol in postharvest treatments

Ethanol is widely used as a disinfectant or sanitiser in pest and disease management of different fruit types in organic crop production as approved by the USDA. Immersion of grape in ethanol (35% v/v) along with hot water treatment (50°C) can reduce the postharvest grey mould development and can be applied as an alternative to synthetic fungicides (Gabler et al., 2005). Exposure to ethanol vapour at the rate of 2 mL kg^{-1} found to be as effective as sulphur dioxide pads in controlling stem browning and rot development in 'Chasselas' table grapes (Chervin et al., 2005). It has been reported by Romanazzi et al., (2007) that immersion of table grapes into 10 or 20% ethanol in combination with 0.5% chitosan significantly controlled the development of postharvest fungal disease, grey mould, development up to 94% compared to untreated berries.

Ethanol has potential application in postharvest industries to retard flower senescence and to regulate (either to promote or inhibit) fruit ripening. Podd and Staden (1998) reviewed the role of ethanol in inhibiting flower senescence and fruit ripening. The external application of 4% ethanol can increase the longevity of vase-life of carnation cut-flower and 02-0.6% ethanol can delay ripening of certain fruit such as tomato. Ethanol inhibits and reduces ethylene production by preventing the conversion of ACC to ethylene and subsequently, delay fruit ripening. However, ethanol does not affect the conversion of ACC to ethylene in some fruit like grape berries. It also reduced the respiration rate of grape, tomato and carnation and delay the lycopene formation intomato (Saltveit, 1989). Podd and Staden (1998) concluded that ripening and senescence inhibition of ethanol is through the conversion of ethanol to acetaldehyde by alcohol dehydrogenase. Tzortzakis and Economakis, (2007) also reported that

ethanol can retard ripening in some fruit such as tomato whilst it stimulates ripening in others like kiwi. Pesis (2005) reported that application of ethanol can induce maturity in fig, can remove astringency in persimmon and can increase anthocyanin contents in grapes. However, the effect may vary depending on cultivar, maturity stage, concentration and time of exposure. Ritenour et al., (1997) demonstrated that 80% ethanol vapour exposure delayed fruit softening and ripening of avocado but with the development of flesh and skin browning at the recommence of ripening. Ritenour et al., (1997) also studied the effect of ethanol on ripening of different climacteric fruits including nectarine, peach and plum. They found that ethanol vapour treatment (≤ 6 mL kg^{-1} fruit) for 6 h, which delayed the ripening of tomatoes (Saltveit et al., 1992), failed to inhibit ripening of all the tested climacteric fruits, while direct injection of ethanol into musk melon or honeydew melon retained firmness. The proficiency of ethanol to regulate ripening in climacteric fruits is influenced by genetic factors, duration of exposure, concentration and application method.

2.7.3. Cyclodextrins

2.7.3.1 Properties of cyclodextrins

Cyclodextrins are naturally-produced cyclic oligosaccharides. Their unique molecular structure with the inner hydrophobic cavity and the outer hydrophilic surface enables them to encapsulate several hydrophobic guest molecules allowing the guests to be more stable and soluble (Del Valle, 2004). The C-H bonds and the ether functional groups of the molecules are located on the ring pointing inward to produce a hydrophobic cavity while the external surfaces are hydrophilic due to the hydroxyl groups (Valente and Soderman, 2014). The guest molecule is temporarily enclosed inside the hydrophobic cavity of the cyclodextrin molecule either by the Van der Waals binding forces, hydrophobic or dipole-dipole interactions (Consonni et al., 2004). This complexation modifies the physiological properties of the guest molecule such as enhancing the solubility and controlling the volatility of the guest molecule (Valente and Soderman, 2014). Although there are various forms of cyclodextrins depending on the glucose units present, only three major cyclodextrins (Fig. 2.12): α- (six glucose units), β- (seven glucose units) and γ– (eight glucose units) are commonly used (Del Valle, 2004). Their chemical and physical properties are listed in Table 2.1.

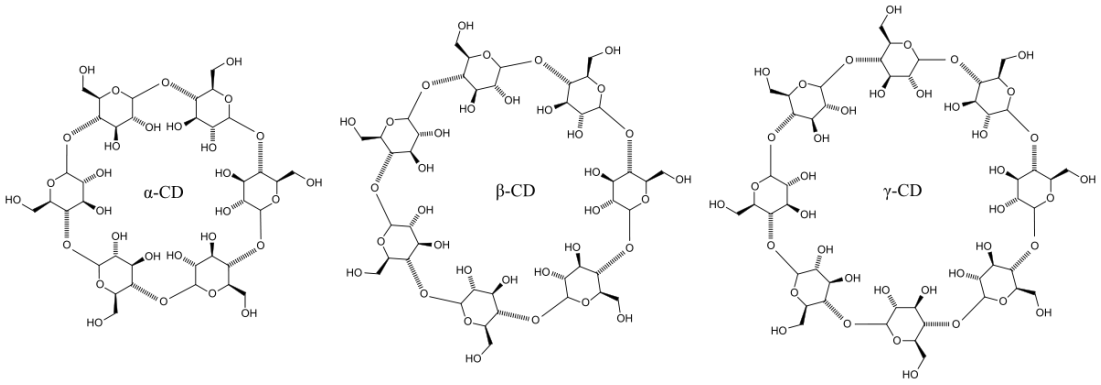


Fig. 2. 12 The structure of α, β and γ cyclodextrins. (Source: Del Valle, 2004)

Table 2. 1 Chemical and physical properties of cyclodextrins

Property	**α-Cyclodextrin**	**β-Cyclodextrin**	**γ-Cyclodextrin**
Number of glucopyranose units	6	7	8
Molecular weight (g/mol)	972	1135	1297
Solubility in water at 25°C (%, w/v)	14.5	1.85	23.2
Outer diameter (Å)	14.6	15.4	17.5
Cavity diameter (Å)	4.7_5.3	6.0_6.5	7.5_8.3
Height of torus (Å)	7.9	7.9	7.9
Cavity volume ($Å^3$)	174	262	427

Source: Del Valle, (2004)

2.7.3.2 Applications of cyclodextrins in postharvest treatments

Cyclodextrins are effective adjuvants for the controlled release of agrochemicals by enhancing their performance and extending the shelf-life of the active compounds (Kenawy and Sherrington, 1992), by increasing solubility (Kim et al., 2008) and by stabilising volatile compounds (Almenar et al, 2007a). Cyclodextrins are extensively used in postharvest regulations of horticultural crops by encapsulating the guest compounds such as ethylene (Ho et al., 2011a and 2011b; Ho and Bhandari, 2016), carbon dioxide (Neoh et al., 2006; Ho et al., 2016) and 1-MCP (Daly and Kourelis, 2001; Seglie et al., 2011a). They are also applied to stabilise the volatile compound acetaldehyde in order to achieve their best performance as fungicide and as sensory quality enhancer during postharvest treatments (Almenar et al, 2007b). They have been used to solubilize poorly water-soluble secondary plant metabolites, as flavonoids (Kim et al., 2008). Cyclodextrins are also used to overcome the unpleasant taste of

plant-based phytochemical compounds while preserving their bioactivity and bioavailability in the functional food industry (Fang and Bhandari, 2010).

The main advantage of cyclodextrin complexations in the postharvest industry is enhancing the efficient delivery of the treated active compounds ethylene, ethylene antagonists, pesticides and fungicides. An overview of the uses of cyclodextrins in postharvest management of horticultural crops is listed in Table 2.2.

Table 2. 2 The use of cyclodextrins in postharvest management of horticultural crops.

Sr. No.	Crops	Purpose	Active ingredient and form of CD	Inference	Reference
1.	Carnations (*D. caryophyllus* L.) var. 'Idra di Muraglia'	Anti-ethylene treatment	1-MCP (0.25 µL L^{-1}) encapsulated in β-cyclodextrin nanosponges	Reduce ethylene production, Maintain original petal colour	Seglie et al. (2011 b)
2.	Banana (*Musa spp*., group AAA, subgroup Cavendish, cultivar Williams)	Anti-ethylene treatment	1-MCP (300 nmol mol^{-1}) followed by Ethylene-cyclodextrin complex (1200 or 2400 nmol mol^{-1})	Delay ripening with regular pattern	Botondi et al. (2014)
3.	*Anemone coronaria* L. multicolour, *Ranunculus asiaticus* L. 'Minou Abrown', *Helianthus annuus* L. 'Sunrich Orange', *Rosa hybrida* L. 'Jupiter', *Paeonia lactiflora* Pall. 'Sarah Bernhardt', and *Papaver nudicaule* L. multicolour	Anti-ethylene treatment	1-MCP (0.25 µL L^{-1}) encapsulated in β-cyclodextrin nanosponges	Enhance the effectiveness of 1-MCP in prolonging the vase-life of all tested cut flower species	Seglie et al. (2013)
4.	Mango (*Mangifera inidca* L.) cv. 'Calypso'	Inducing Ethylene	Ethylene + α-cyclodextrin powder	Enhance ripening up to 4 d	Ho et al. (2016)
5.	Carnations (D. *caryophyllus* L. 'Idra di Muraglia'	Anti-ethylene treatment and Fungicides	1-MCP (0.25 µL L^{-1}) encapsulated in β-cyclodextrin nanosponges	Reduce the development of grey mould disease (by reducing the ethylene)	Seglie et al. (2012)

	Crops	Purpose/Class	AI and form of CD	Inference	Reference
6.	Cress, Onion, Lettuce, Tomato	Herbicide	Sesquiterpene lactones-cyclodextrins complex	Increase the solubility and enhanced the activity of active compounds	Cala et al. (2017)
7.	Blueberry	Fungicides	Acetaldehyde-β-cyclodextrin	Reduce the postharvest fungal growth	Almenar et al. (2007b)
8.	Red-fleshed grapefruit (*Citrus paradisi* var. macf.) cv. Star Ruby, Blood oranges [*Citrus sinensis* var. (L.) osbek] cv. Tarocco, and lemons [*Citrus limon* var. (L.) burm] cv. Di Massa	Fungicides	Imazalil (250 mg L^{-1}) + β-Cyclodextrin	Suppress the postharvest decay caused by Penecillium in citrus	Schirra et al. (2002)

CHAPTER 3

General materials and methods

3.1 Source of fruit and experimental conditions

Seven varieties of stone fruit with different harvest seasons (i.e. early, mid and late maturing cultivars), which are commercially being produced in Western Australia (Table.3.1), were used to investigate the effects of different formulations of ethylene antagonists on stone fruit postharvest quality and physiology under various storage conditions. All the fruit were generously provided by the Eastwind Farm (33°47'02.4"S 115°57'47.2"E), Western Australia, except 'Flavor Fall' pluot which was supplied by the Della Pollina orchard (32°02'08.3"S 116°09'06.5"E), Pickering Brook, WA. The detailed information on tree age, rootstock and spacing (plant-plant x row-row) of the tested cultivars are described in (Table 3.1). Fruit trees are trained with a double leader training system. The orchard management practices, i.e. irrigation, fertilizer application and pest and disease management, are according to the guidelines of the Department of Agriculture and Food, the Government of WA. The commercially matured fruit which was uniform in size, free from pest and disease and mechanical injuries were selected. The fruits were packed in plastic baskets as shown in Fig. (3.1) and transported to the Horticulture Research Laboratory, Curtin University, Perth, Western Australia by air-conditioned truck. All the experiments were carried out within 36 hours after harvest.

3.2 Chemicals used in formulations of ethylene antagonists

The ethylene antagonists 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) were supplied by Dr Alan Payne, School of Molecular and Life Sciences, Curtin University. The commercial ethylene antagonist 1-methylcyclopropene (1-MCP), known as SmartFresh^{TM}, was purchased from AgroFresh Solutions Inc., USA. The adjuvants; ethanol (FSBE/0665DF/15, ThermoFisher Scientific, USA), Ecotoric Tween® 20 Labchem (AJA2509-500ml, ThermoFisher Scientific, USA) and β-cyclodextrin (C4805, Sigma-aldrich®, USA) were used for the preparation of the different formulations of ethylene antagonists BC and NC.

Table 3. 1 Tree age, rootstock and spacing of the tested cultivars.

No.	Fruit	Tree age	The rootstocks	Spacing	Row direction
1.	Plum cv. 'Fortune'	20 years	Plum	1.5 m x 4 m	North to South
2.	Plum cv. 'Tegan Blue'	25 years	Peach	1.5 m x 4 m	North to South
3.	Plum cv. 'Angeleno'	20 years	Plum	1.5 m x 4 m	North to South
4.	Nectarine cv. 'Diamond Bright'	20 years	Peach	1 m x 4 m	North to South
5.	Nectarine cv. 'Ruby Diamond'	22 years	Peach	1 m x 4 m	North to South
6.	Peach cv. 'Princess Time'	2.5 years	Peach	1.5 m x 4 m	North to South
7.	Pluot cv. 'Flavor Fall'	6 years	Plum	1.8 m x 3.8 m	North to South

Table 3. 2 Chemical name, formula, structure and molecular weight of ethylene antagonists tested in the present research

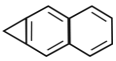
No	Chemical name	Formula	Structure	MW (g)
1.	1-methylcyclopropene (1-MCP)	C_4H_6		54.09
2.	1*H*-cyclopropabenzene (BC)	C_7H_6		90.1
3.	1*H*-cyclopropa[*b*]naphthalene (NC)	$C_{11}H_8$		140.2

Fig. 3. 1 Freshly harvested 'Tegan Blue' plums sourced from the Eastwind orchard

3.3 Preparation of different formulations of ethylene antagonists

3.3.1 Preparation of spray treatments

The concentrations for both BC and NC in all aqueous solutions for spray treatments were 2 µM. The spray solutions were prepared by following the procedure of Choi et al., (2008) with some modifications as follows.

(i) Aqueous solutions of BC and NC with distilled water only (BCA and NCA): For BCA, the required amount of BC was added into the volumetric flask, then, the final volume was made into 250 mL by adding distilled water. Spray solution for NCA was prepared following the same procedure as for BCA.

(ii) Aqueous solutions of BC and NC with 0.02% Tween® 20 (BCT and NCT): For BCT, the required amount of BC stock solution was first added into the volumetric flask, then, 50 µL of Tween® 20 was added into the prepared stock. After that, distilled water was added gradually till the final solution of 250 mL was made. Spray solution for NCT was prepared following the same procedure as for BCT.

(iii) Aqueous solutions of BC and NC with 5% β-Cyclodextrin (BCD and NCD): A β-cyclodextrin (5 %) stock solution was prepared by dissolving the 1.25 g of β-cyclodextrin in 250 mL of distilled water at 60 °C using a thermomagnetic stirrer until the β-cyclodextrin powder is completely dissolved. For BCD, the required amount of BC stock solution was added into the volumetric flask. Then, the final volume was made into 250 mL by adding the prepared β-cyclodextrin (5%) solution. Spray solution for NCD was prepared following the same procedure as for BCD.

(iv) Aqueous solutions of BC and NC with 5% Ethanol (BCE and NCE): For BCE, the required amount of BC stock solution was first added into the volumetric flask. Then, 12.5 mL of ethanol was added into the prepared stock and then mixed thoroughly. The final volume was made into 250 mL by adding distilled water. Spray solution for NCE was prepared following the same procedure as for BCE.

The fruit were spread into one layer on a plastic tray and sprayed thoroughly with BC or NC solutions using a hand sprayer (500 mL). Following the spray treatments, the fruit were kept in the air-tight plastic container for 18 h to generate the same storage condition as the fumigation treatments. After 18 h, the fruit were taken out of the

container in an open space and arranged replication wise in the labelled cardboard boxes according to the experimental designs.

3.3.2 Preparation of fumigation treatments

The fumigation treatments were conducted by following the method of Khan (2007). For all the fumigation treatments, 1 µM concentration of BC and NC were applied for 18 h in air-tight plastic containers (60 L). Portable fans were used to generate the homogenous fumigant concentration inside the containers. About 25 g of soda-lime was kept inside the containers by using Petri dish to absorb the carbon dioxide produced by the fruit.

(i) Fumigation of BC and NC (BCF and NCF): The fruit were kept inside the container along with the portable fan to circulate the air inside the container and a Petri dish (250 g) of soda-lime to absorb the carbon dioxide generated by the fruit (Fig. 3.2). The required amount of BC or NC (1 µM) for 60 L plastic container was placed in a petridish containing a filter paper. The Petri dish was filled with 5 mL of ethanol to upsurge the volatility of BC or NC. The container was closed tightly as soon as the BC or NC was applied and kept for 18 h (Fig. 3.3).

(ii) Fumigation of 1-MCP: 1-MCP was applied according to the direction of the SmartFresh® for 18 h in an air-tight plastic container (Khan and Singh, 2008).

After 18 h of storage, the treated fruit were taken out of the container in an open space and arranged replication wise in the respective cardboard boxes, which were labelled previously according to the experimental designs (Fig. 3.4). The initial weight before storage and the final weight at the end of storage were taken for each replication to calculate the physiological weight loss. The labelled cardboard boxes were then placed in the cold room at 0±1°C and 90±5% RH.

3.4 Storage conditions

For the ambient condition, the fruit were kept under the normal atmospheric condition at 21±1.5°C with 60±5 % relative humidity. For the cold storage condition, the storage room was maintained at 1°C with 90±5 % RH. To create the modified atmospheric packaging (MAP) storage condition, fruit were kept in the commercial MAP bags

(LifeSpan®, AMCOR Packaging, Ptv. Ltd., Australia) which are specially designed for plums, and kept at 1°C after treating with ethylene antagonists. The MAP bags were sealed tightly after keeping for 4 h at 1°C when the temperature inside the bag and in the storage room was in equilibrium stage in order to avoid condensation (Khan and Singh, 2008). For all the experiments, the temperature and relative humidity of the storage room were monitored using Tinytag*Plus* Gemini Data Loggers (Gemini Dat Loggers, Ltd., UK).

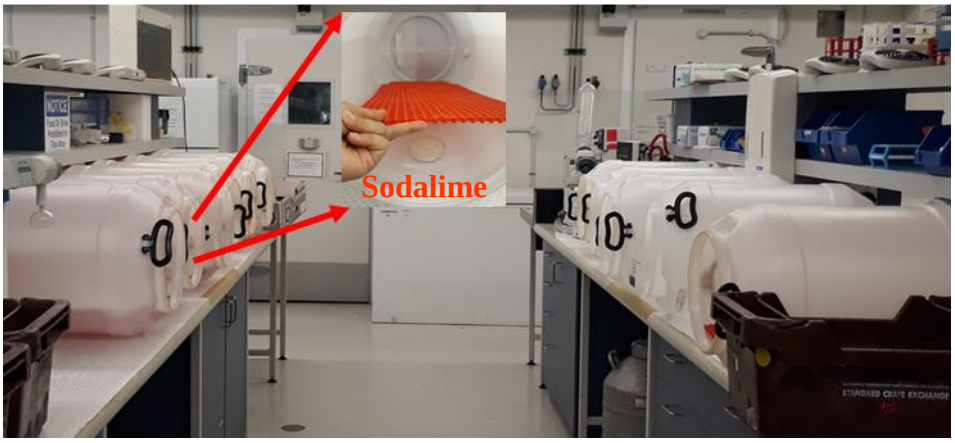


Fig. 3. 2 Plastic containers prepared for treating the fruit for 18 h

Fig. 3. 3 Tightly closed plastic containers with the treated fruit

Fig. 3. 4 Opening the fumigated containers in open space after 18 h of storage

3.5 Determination of fruit physiological parameters

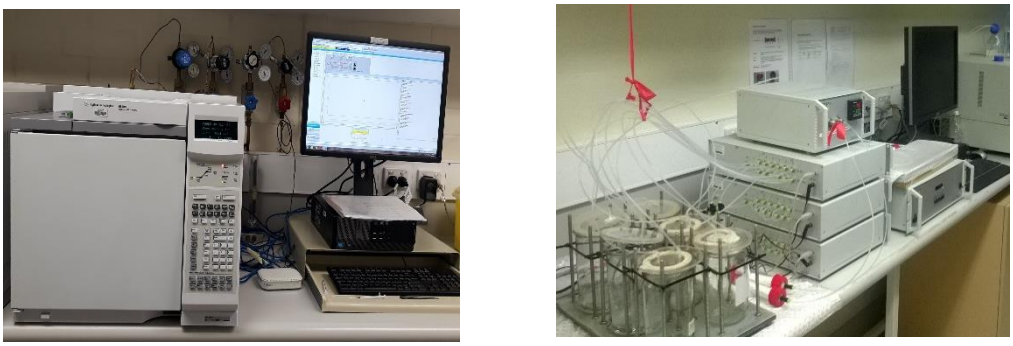
3.5.1 Ethylene production

For the climacteric fruit type ‘Fortune’ and ‘Tegan Blue’ plums, ‘Diamond Bright’ and ‘Ruby Diamond’ nectarines, and ‘Princess Time’ peaches, the ethylene production rate was determined by following the detailed method of Khan and Singh (2008) using Agilent 6890N Network Gas Chromatogram, (Agilent Technologies, Santa Clara, USA) (Fig. 3.5 A). The fruit (3 fruit per replication) were sealed for 1 hour in airtight jars (1 L) fitted with a rubber septum (Fig. 3.5). 1 mL of headspace gas sample was taken from the jar using a syringe and injected into the gas chromatograph. The ethylene concentration of the sample was standardised by comparing the retention time of the standard ethylene (1.15 ± 0.06 ppm in N_2, BOC Gases, Australia Ltd., Perth, Australia) (Fig. 3.8). The ethylene production rate was calculated by the following formula.

$$\text{Ethylene production} = \frac{\text{ethylene conc. by GC } (\mu L\ L^{-1}) \text{ x headspace volume (L)}}{\text{Fruit weight (kg) x incubation time (h)}}$$

For the suppressed-climacteric fruit types ‘Angeleno’ plum, and ‘Flavor Fall’ pluot, the ETD-300 ethylene detector (Sensor sense B.V, Nijmegen, The Netherlands) (Fig. 3.5 B) was used to detect ethylene production rate following the method described by Azzu (2016). The fruit (3 fruit per replication) were weighed and then sealed in the

individual air-tight jars (Fig. 3.5 B). The headspace gas from the jars was pumped to the ethylene detector passing through a catalyser which removes hydrocarbons in the sample gas, and a valve controller which allowed computerised multi-sample detection (six samples simultaneously) (Datasheet valve control box, Sensor Sense). Prior to entering the ethylene detector, the sample gas was purified again by passing into soda-lime and calcium chloride (carbon dioxide and water scrubbers, respectively). The ethylene production rate was detected by using the continuous flow method at the flow rate of 4 L per h for 20 min (Fig. 3.8). The data were automatically analysed by the software supplied along with the valve controller (Sensor sense, Nijmegen, The Netherlands). The ethylene production rate was expressed as nmol kg^{-1} h^{-1}.



(A) (B)

Fig. 3. 5 (A) The Gas Chromatogram (Agilent 6890N) and (B) ETD-300 (Sensor Sense) ethylene detector for determination of ethylene

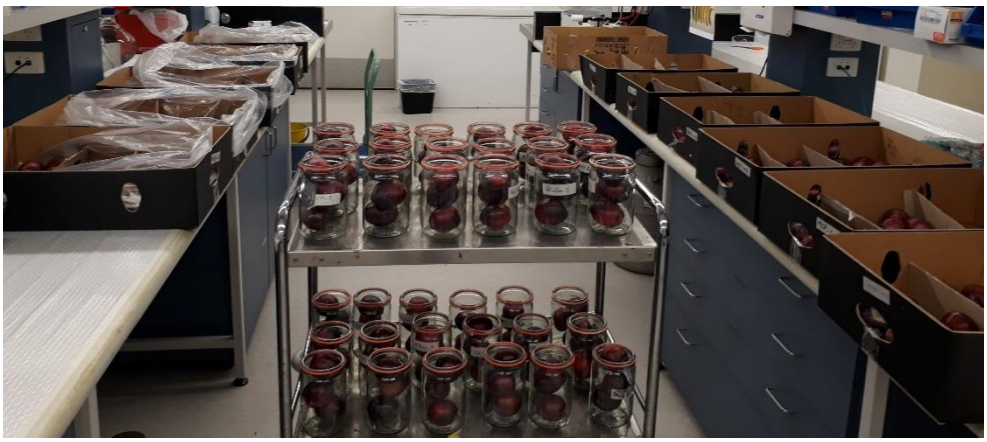


Fig. 3. 6 The fruit selected for determination of ethylene and respiration

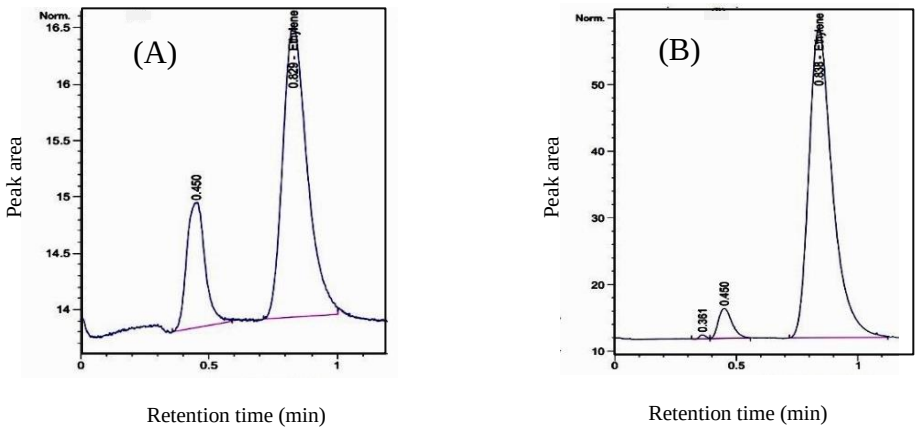


Fig. 3. 7 The ethylene peaks of standard (A) and sample fruit ‘Princess Time’ peach (B) detected by GC

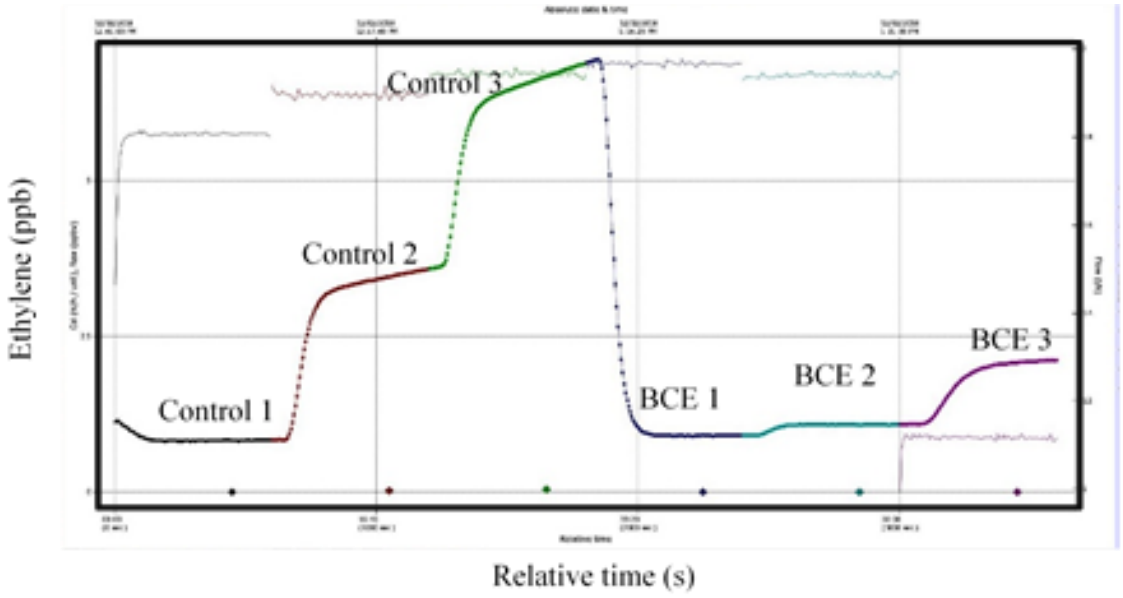


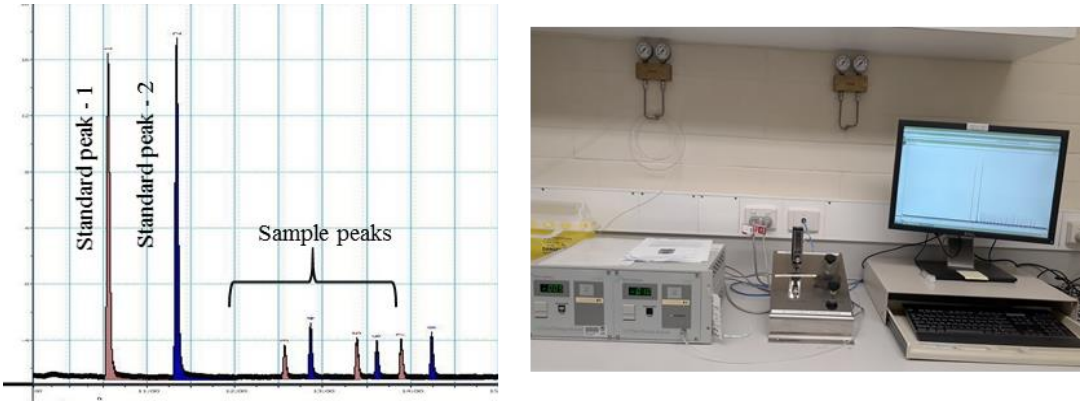
Fig. 3. 8 The ethylene production of ‘Tegan Blue’ plum detected by ETD-300 using continuous flow method

3.5.2 Respiration rate

Respiration rate was determined by using the gas analyser (1450 series Gas Analyser, Servomex, UK) following the method described by Khan and Singh (2008). The fruit sample (3 fruit per replication) were weighed and then, sealed tightly for 1 hour in the

1 L jars fitted with a rubber septum (Fig. 3.9). The 2 mL volume of headspace gas was used to determine the respiration rate by quantifying the concentration of carbon dioxide (CO_2) in the sample using the peak area of the standard CO_2 gas (8.31 ± 0.17 % in N_2, BOC Gases, Australia Ltd., Perth, Australia) (Fig. 3.9). The respiration rate was calculated as the concentration of carbon dioxide in the sample gas according to the following formula. The respiration rate was mentioned as mmol CO_2 kg^{-1} h^{-1}.

$$\text{Respiration rate } (CO_2\ kg^{-1}\ h^{-1}) = \frac{\text{Volume of } CO_2\ \text{(mL)}}{\text{Fruit weight (kg) x incubation time (h)}}$$



(A) (B)

Fig. 3. 9 The detection of carbon dioxide peaks in the fruit sample (A), by using a gas analyser (1450 series, Servomex) (B).

3.5.3 Physiological weight loss

The physiological weight loss of fruit was calculated following the method of Azzu (2016). To calculate the percent weight loss, initial weight and final weight of each replication for individual treatment were recorded before keeping in the storage room and at the end of the storage period (Fig. 3.10). The physiological weight loss was calculated by using the following formula.

$$\text{Weight loss}(\%) = \frac{(\text{Initial weight-Final weight})}{\text{Initial weight}} \text{ x } 100$$

Fig. 3. 10 Weighing the fruit of each replicate to calculate physiological weight loss

3.5.4 Fruit firmness

The fruit firmness was determined by following the method previously described by Khan and Singh (2008) using the texture analyser (TPA Plus, AMETEK Lloyd Instruments, United Kingdom) fitted with 8 mm probe (Fig. 3.11). Ten individual fruit per each replication were used to estimate the firmness. The two opposite sides of the fruit-cheek were peeled approximately to 2.5 mm depth. The firmness was tested two times by puncturing the fruit flesh with a trigger force of 1 N to the depth of 8 mm at 100 mm s^{-1} speed. The firmness was automatically recorded by the Nexygen® software which was interfaced to the textural analyser and the fruit firmness was expressed in N.

3.6 Determination of fruit quality parameters

One longitudinal slice from each fruit (ten fruits per replication) was taken without peel, cut into small cubes and mixed to get the homogenous sample for each replication. The exact weight of sample for the respective observations: 5 g each for individual sugars and organic acids, 20 g for total phenols, 5 g for ascorbic acid and 1 g for antioxidants were kept at -20°C until the fruit quality analyses were undertaken.

Fig. 3. 11 Testing the fruit firmness of 'Diamond Bright' nectarines by using Texture Analyser (TPA Plus, AMETEK Lloyd Instruments).

3.6.1 Soluble solids content (SSC), titratable acidity (TA) and SSC: TA

The portable digital refractometer (Atago-Palette PR 101, Atago Co., Itabashi-Ku, Tokyo, Japan) was used to determine the percent SSC content by following the procedure previously described by Khan and Singh (2008). The longitudinal sections from the individual fruit (ten fruits per each replication) were peeled, cut and then, made juice by using a fruit juicer (The Froojie Fountain Juicer, Breville, Australia). The juice was used to take three SSC readings for each replicate. The values were expressed as percent Brix.

The same fruit juice taken for SSC was used to quantify the amount of titratable acidity (malic acid equivalent) by following the method of Khan and Singh (2008). To determine TA, 10 mL of fruit juice was first diluted with 20 mL of distilled water. The 5 mL of diluted juice was titrated against 0.1 N NaOH. Phenolphthalein was used to indicate the endpoint (pale pink colour) of the titration process. The volume of NaOH was used to calculate the TA according to the following formula.

$$\text{TA \% (malic acid equivalent)} = \frac{0.0067 \times \text{Vol. of NaOH (mL)} \times \text{Total vol. (mL)} \times 100}{\text{Vol. of Juice (mL)} \times \text{Vol. of aliquot (mL)}}$$

Following the method described by Khan and Singh (2008), the ratio of SSC:TA was calculated by dividing the value of SSC by the respective value of TA.

3.6.2 Individual sugars and organic acids

The HPLC analytical grade standards of individual sugars (glucose, sucrose, and fructose), sugar-alcohol (sorbitol) and organic acids (malic, citric, fumaric and succinic) were purchased from Sigma-Aldrich, Sydney, Australia. All the standards and samples for sugar and organic acid analysis were prepared by using Milli-Q degassed water, which was purified through the water purification system and the vacuum filtration degasser (Millipore, Bedford, USA). The quantification of sugars and organic acids was undertaken by using a reversed-phase high performance liquid chromatography (HPLC) (Binary HPLC Pump, Waters 1525, Milford Corp., USA) following the detailed procedure of Usenik et al. (2008) with some modifications.

(i) Preparation of samples: Just prior to analysis, the previously prepared sample (5 g) were homogenised by using an electronic homogeniser (Heidolph Homogeniser, DIAX 900, Sigma Aldrich, Australia) and then, diluted up to 50 mL with Milli-Q degassed water. The diluted sample was centrifuged for 15 min at 12857 *g* by using the refrigerated centrifuge (Eppendorf Centrifuge 5810R, Hamburg, Germany). 1 mL of aliquot was filtered by using a 0.22 μm nylon syringe filter (Thermo Fisher Scientific Pty Ltd., Australia) and kept in the 1 mL clear glass HPLC vial shell with polyethylene plug (ThermoFisher Scientific Pty Ltd., Australia).

(ii) Analysis of individual sugars and organic acids: The prepared standards and samples were injected automatically by an autosampler (Waters 717plus, Milford Corp., MA, USA). Individual sugars were detected by the Refractive Index Detector (Waters 2414, Milford Corp., MA, USA), while individual organic acids were determined by a Dual λ UV absorbance detector (Water 2487, Milford Corporation, USA) at 214 nm. The Milli-Q degassed water and 0.1 N sulphuric acid were applied as mobile phases for sugar and organic acid assay, respectively. The Fast Carbohydrate Analysis column (100 x 7.8 mm) and Organic Acid Analysis column (300 x 7.8 mm) (Aminex® HPLC columns, BIO-RAD Laboratories, Inc., Hercules, USA) were used for separation of individual sugars and organic acids, respectively. The flow rate was maintained at 0.6 mL min^{-1} during the HPLC analysis process. The concentrations of individual sugars and organic acids in each sample were quantified by standardising the retention time of the standard curves (Fig. 3.12 and 3.13) using the Breeze® 2 software and the values were expressed as g kg^{-1}.

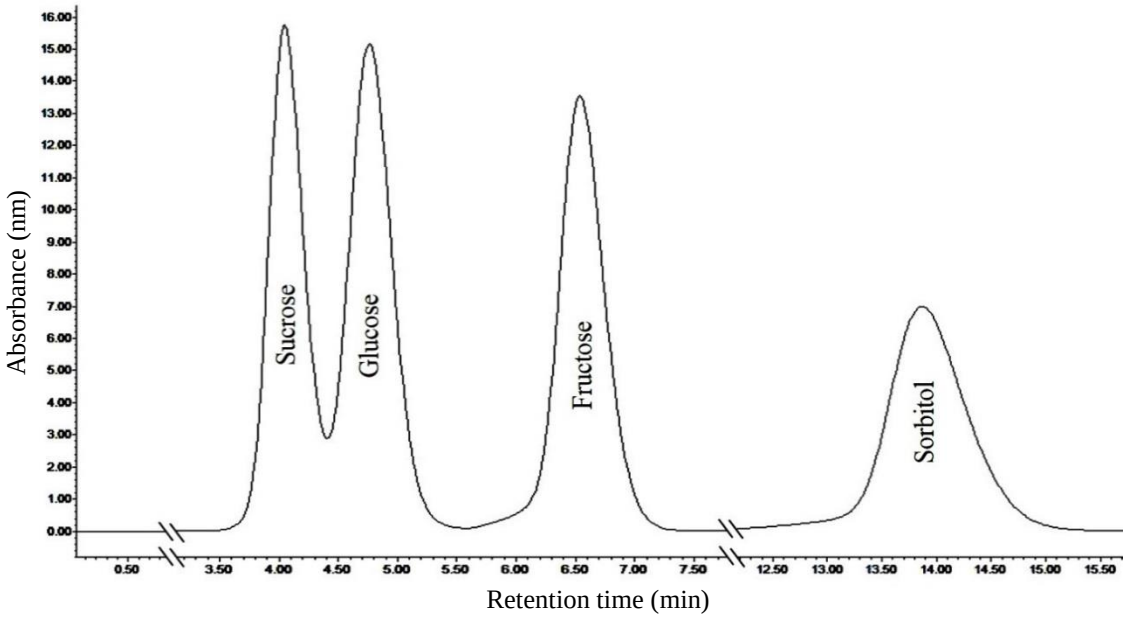


Fig. 3. 12 The individual sugars and sugar-alcohol standard profiles separated by HPLC

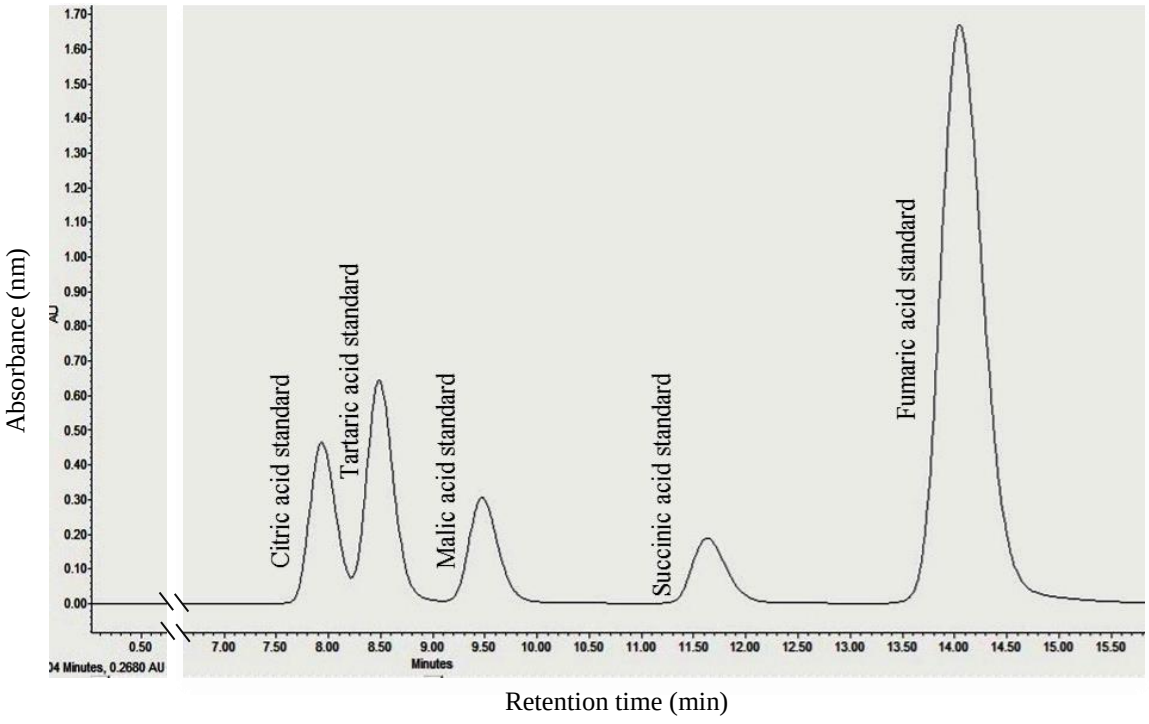


Fig. 3. 13 The individual organic acids standard profiles separated by HPLC

3.6.3 Determination of total phenols

The determination of total phenols content in the fruit pulp was undertaken by following the procedure of Cantin et al. (2009) with some modifications. Methanol, sodium carbonate, Folin reagent and gallic acid standard (analytical grade) were purchased from Sigma Aldrich, Sydney, Australia.

Extraction of phenols: The prepared sample (20 g) was mixed with 15 mL of extraction solution, 80% methanol (v/v) and then, homogenised for 30 s. The homogenised sample was then sonicated for 15 min by using an ultrasonic cleaner (Soniclean Pvt. Ltd., Therbaton, South Australia, Australia). Following the sonication, the samples were centrifuged for 15 min at 10,000 *g* at 5 °C. The supernatant was collected in a 100 mL centrifuge tube and the pulp residue was re-extracted again following the same procedure. The two aliquots were combined, and the final volume of 60 mL was made by adding distilled water.

Total phenols assessment: The extracted aliquot of 50 µL was mixed with 3 mL distilled water in the presence of 250 µL of Folin reagent which was diluted in distilled water (1:2). The aliquot mixture was kept in the dark for 5 min prior to adding the 250 µL of 7% sodium carbonate (Na_2CO_3) solution and 1450 µL of distilled water to get a final mixture of 5 mL. The final mixture was kept in the dark for 90 min. Subsequently, the absorbance value was measured by using a spectrophotometer (6405 UV/visible (190-1100 nm), Jenway, Dunmow, Essex, UK) at the wavelength of 750 nm. The total phenol content was calculated from a calibration curve using gallic acid as a standard. The total phenol contents were expressed in term of gram gallic acid equivalents per kilogram (g GAE kg^{-1}) fresh pulp weight.

3.6.4 Determination of ascorbic acid

The ascorbic acid content in the fruit pulp was determined by following the method described by Vithana (2017). The required chemicals: metaphosphoric acid (MPA), ethylenediaminetetraacetate acid (EDTA), Folin reagent and L-ascorbate (analytical grade) were purchased from Sigma-Aldrich, Sydney, Australia.

Preparation of extraction solution: For 6% MPA solution, 60 g of metaphosphoric acid crystal was dissolved in 1 L distilled water with the presence of 1.8 g EDTA. For 3 %

MPA solution, the 6 % MPA solution was diluted into 1:2 concentration with distilled water. The Folin reagent solution was prepared by diluting the concentrated Folin reagent with distilled water into 1:5 concentration.

Ascorbic acid assay: 5 g of prepared sample was homogenised in 20 mL of extraction solution (6 % MPA) by using a homogeniser. The homogenised extract was centrifuged for 20 min at 5000 *g*. After centrifuging, a mixture of solution which contained 400 µL of supernatant, 200 µL of 3 % MPA solution, 200 µL of diluted Folin reagent and 1400 µL of distilled water was prepared and kept in the dark for 10 min. The absorbance of the prepared solution was measured at 760 nm by using the spectrophotometer. The ascorbic acid content was estimated by calibrating with the standard curve of L-ascorbate and expressed as g kg^{-1}.

3.6.5 Determination of total antioxidant capacity

The total antioxidant capacity in the fruit pulp was estimated by checking the free radical scavenging capacity using the DPPH method. The estimation was done by following the detailed procedure of Vithana (2017) based on the method of Brand-Williams et al. (1995). The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical, sodium fluoride (NaF) and Trolox (6-hydroxy-2,5,7,8-tetramethylchoman-2-carboxylic acid) were purchased from Sigma-Aldrich, Sydney, Australia.

Preparation of DPPH free radical solution: To prepare the DPPH stock solution, 24 mg of DPPH powder was dissolved in 100 mL of 100% methanol. The stock solution was diluted into 1:4 concentration with 100% methanol. The diluted solution was adjusted to get the absorbance value 1.1 at 515 nm, either by adding methanol (if the absorbance was > 1.1) or stock solution (if the absorbance was < 1.1), by using the spectrophotometer.

Extraction of total antioxidant compounds: The prepared sample (1 g) was mixed with 10 mL of sodium fluoride (NaF) extraction solution, which was prepared by mixing 84 mg of NaF in 1 L of 80% methanol, homogenised for 30 s and then, centrifuged at 4°C for 20 min at 10,000 rpm. The required amount of supernatant was mixed with 1900 µL of diluted DPPH solution and kept in the dark for 15 min, and then, the absorbance value was recorded at 515 nm by using the spectrophotometer. This spectrophotometric assay was repeated until the absorbance value was in the range of

0.6-0.7 at 515 nm. The recorded amount of supernatant and the value of absorbance were used in the calculation of antioxidants activity which was calibrated by using the standard curve of Trolox. The total antioxidant activity in the fruit pulp was expressed in term of mmol Trolox equivalent antioxidant activity per kg (mmol TEAC kg^{-1}).

3.6.6 Determination of total anthocyanin content

The total anthocyanin content was examined based on the method of Siegelman and Hendricks (1958) by following the detailed procedure of Whales and Singh (2007).

Extraction of anthocyanins: The prepared peel sample (1 g) was mixed with 10 mL of extraction solution containing 95% aqueous methanol and concentrated HCl in the ratio of 97:3 (v/v) and homogenised for 1 min. The extracted solution was then kept in the dark at 2-4 °C overnight and the decanted solution was centrifuged for 20 minutes at 3963 *g*.

Anthocyanin assay: The supernatant of centrifuged sample solution was checked at 530 nm wavelength to determine the anthocyanin absorbance value using the spectrophotometer. The molar extinction coefficient values of idaein chloride (Cyanidin-3-glucoside) which was used as a standard for the calculation of total anthocyanin is $3.43x10^4$ (Siegelman and Hendricks, 1958). The total anthocyanin content was expressed as gram Cyanidin-3-glucoside kg^{-1} of fresh peel weight.

3.7 Statistical analysis

Data were analysed by one-way or two-way analysis of variance using Genstat software (13^{th} edition), Genstat release 13.1, VSN International Ltd., UK. Least significant differences (Fisher's LSD) were calculated following the significant F test ($P \leq 0.05$). The treatment means were compared by using the Duncan's Multiple Range Test. The analysed results are tabulated as means± standard error of means.

CHAPTER 4

Effect of different formulations of 1*H*-cyclopropabenzene on ethylene production and quality of plums (*Prunus salicina* Lindell cvs. 'Fortune', 'Tegan Blue' and 'Angeleno') under cold storage

Summary

The effectiveness of different formulations of 1*H*-cyclopropabenzene (BC) on postharvest physiology and quality parameters of plum were investigated using three commercial Japanese plums 'Fortune', 'Angeleno' and 'Tegan Blue'. The plums were treated with different formulations; BC fumigation (1 µM) for 18 h at 20±1°C and the spray treatments with BC aqueous solution (2 µM) containing 5 % ethanol, BC aqueous solution (2 µM) containing 0.02 % Tween® 20, BC aqueous solution (2 µM) containing 5 % β-cyclodextrin and BC aqueous solution (2 µM) only at 20±1°C. The untreated fruit were considered as control. All fruits were stored at 0±1°C (90±5% RH) for 25 and 40 d following the respective treatments. Ethylene production and postharvest fruit quality parameters were evaluated immediately after removal from the respective cold storage. BC formulations significantly suppressed ethylene production, reduced weight loss, SSC % and SSC:TA maintaining higher fruit firmness and TA contents depending on the cultivar and the adjuvants applied. After 40 d cold storage, ethylene production was lower, regardless of cultivars and BC formulations, as in response to low temperature. The fruit quality parameters such as individual sugars and organic acids, total phenols, ascorbic acid, antioxidant activity and total anthocyanin contents responded differently to BC formulations depending on cultivars. The effectiveness of BC was more pronounced in BC fumigation, BC solution containing ethanol and BC solution containing Tween® 20 as compared to control and other BC solutions. In conclusion, BC maintained the visual and organoleptic quality of plums up to 40 d under cold storage by suppressing ethylene production. The addition of adjuvants ethanol and Tween® 20 in aqueous spray solutions was found to improve the performance of BC in retarding ethylene production and maintaining postharvest fruit quality.

4.1 Introduction

Japanese plums (*P. salicina* L.) are one of the major *Prunus* species with a diverse range of cultivars cultivated worldwide (Topp et al., 2012). They have been known as the alternative functional food in the human diet due to their reported health benefiting properties (Sahamishirazi et al., 2017). Most of the plum cultivars are climacteric fruit with a rise in ethylene production at the commencement of ripening. This climacteric rise in ethylene production triggers the subsequent ripening processes such as softening, colour development, sugars and organic acids accumulation and the development of flavour and aroma (Khan and Singh, 2008). However, some cultivars such as 'Angeleno', Shiro' and 'Rubyred' do not exhibit this behaviour of ripening. These cultivars are unable to produce a large amount of internal ethylene like normal climacteric cultivars but they responded to exogenous ethylene and exhibit a suppressed-climacteric nature (Abdi et al., 1998; Candan et al., 2008, Manganaris et al., 2008).

Plums are extremely sensitive to ethylene even at the very low concentrations of 0.01-0.1 µL $kg^{-1}h^{-1}$ (Crisosto and Kader, 2000). In response to either endogenous or exogenous ethylene, the ripening of plums and the related processes become accelerated leading to quality deterioration during postharvest handling of fruits (Manganaris et al., 2008; Kader and Yahia, 2011). Due to their high ethylene sensitivity, the storage of plums for domestic supply or the long-distance market is greatly limited (Khan et al., 2018). Cold storage has been the most fundamental method applied to delay ripening, especially for the fruits intended to be transported to long-distance markets and to maintain the postharvest quality of plum (Manganaris et al., 2008; Benichou et al., 2018). Robertson et al. (1991) examined the fruit quality of 'Au-Rubrum' plum such as fruit colour, soluble solids and total sugar contents after storing for 0, 1, 2, 3 and 5 weeks at 0 °C. They found that the plum can be stored for up to 5 weeks without quality deterioration assuring the potential for long distant transport (Robertson et al., 1991). The inhibition of ethylene action at the receptor level is also one of the best approaches to avoid the ethylene-induced ripening acceleration during postharvest handling (Manganaris et al., 2008; Khan et al., 2018). The application with different concentrations of 1-MCP (13, 26 or 39 µL L^{-1}) for 24 h at 20 °C delayed the ripening processes of climacteric and suppressed-climacteric plums by blocking the ethylene action (Abdi et al., 1998). The integrated postharvest

approaches such as 1-MCP in combination with cold storage to slow down ripening and to extend the marketable life of fruit have also been developed and practised successfully (Valero et al., 2003; Benichou et al., 2018).

Recently, Singh et al. (2018) have discovered the application of new ethylene antagonist 1*H*-cyclopropabenzene (BC) can retard the ethylene-induced flower senescence and fruit ripening. Although the chemical structure of BC is different from the current commercial ethylene antagonist 1-methylcyclopropene (1-MCP), its chemical reactivity is similar to that of 1-MCP. Unlike 1-MCP which is a gas, BC is a lot easier to handle because it is partially water-soluble and is stable as a liquid at room temperature for long periods. Preliminary studies showed that the postharvest application of BC as fumigant could block the action of ethylene in Japanese plums, apples and wax flowers thereby slowing down the ripening and senescence processes (Singh et al., 2018). However, the application of fumigants is restricted in some postharvest treatments where the air-tight facilities are unavailable and in open field preharvest applications limiting the broader use of the compound (Sisler, 2006; Manganaris et al., 2007). Water-soluble ethylene antagonists which are easy to handle and can protect the receptors for a longer period upon a single exposure are of great interest (Grichko, 2006).

The solubility of BC is similar to the other aromatic hydrocarbons such as toluene and these hydrocarbons are partially soluble in water at room temperature (Bohon and Claussen, 1951). The addition of adjuvants such as co-solvents ethanol (Farag et al., 1992, Grichko, 2006), non-ionic surfactants (Singh and Khan, 2012) into the spray solutions and inclusion with carrier compound as β-cyclodextrins (Ho, 2013) have already improved the solubility of poorly soluble compounds and gases. They are chemically inert and can facilitate the efficient delivery of the active compounds to the targeted plant parts. Hence, ethanol, Tween® 20 and β-cyclodextrins were applied in the preparation of BC spray solutions in the present study.

The development of water-based spray or dip solutions of BC which can be applied as an alternative to fumigation treatment with upmost efficacy is still lacking and warrants to be investigated. Therefore, the efficacy of different formulations of BC in retarding ethylene production and in maintaining the postharvest quality was investigated using three commercial plum cultivars under cold storage conditions. It

was hypothesised that the antagonistic effect of BC on ethylene production will be enhanced by the addition of the adjuvants in BC solutions and thus, hinder the subsequent ripening-related quality changes of plum after postharvest cold storage.

4.2 Materials and methods

4.2.1 Experimental conditions

The efficacy of different BC formulations on ethylene production and the postharvest quality of Japanese plums (*P. salicina* L.) cvs. 'Fortune', 'Angeleno' and 'Tegan Blue' were examined by conducting three individual experiments under cold storage. All the plum cultivars were sourced from the Eastwind Farm (33°47'02.4"S 115°57'47.2"E), Western Australia. The different formulations of 1*H*-cyclopropabenzene (BC) solutions were prepared by following the procedure of Choi et al., (2008) and Khan (2007) as explained in section 3.3.1.

4.2.1.1 Experiment 1: Efficacy of different formulations of BC on postharvest physiology and quality of plum cv. 'Fortune'

'Fortune' plums with the fruit firmness of 53.1±4.0 N, TSS of 10.4±0.3 and TA of 2.3±0.01 were sourced from the Eastwind Farm on 17th January 2017. The fruit characterised by uniform size without any visual defects were selected for the experiment. The experiment was arranged in a a completely randomised design (CRD) with four replications. The fruit were divided into six lots (120 fruit each) to be exposed to the experimental treatments; (1) BC (2 µM) solution prepared with only distilled water, (2) BC (2 µM) solution prepared with 0.02 % Tween® 20, (3) BC (2 µM) solution prepared with 5 % β-cyclodextrin, (4) BC (2 µM) solution prepared with 5 % ethanol, (5) BC (1 µM) fumigation and (6) without treatments as control. The applications of treatments were conducted at 20±1°C and the detailed procedures were described in Section 3.3.1 and 3.3.2. After treating for 18 h in an air-tight plastic container, each lot was separated into two sub-lots (60 fruit=15x4) in order to keep in cold storage at 0±1 °C and 90±5 % RH for 25 d and 40 d. Before keeping in the cold storage, the initial weight of each replication (15 fruit) was measured. At the end of each cold storage period, the physiological parameters (weight loss, firmness, SSC and

TA) and the quality parameters (individual organic acids and sugars, total phenols, ascorbic acid, total antioxidant capacity and anthocyanin) were determined. The ethylene production and respiration rate were monitored daily during the ripening period at 20±1°C after cold storage.

4.2.1.2 Experiment 2: Effect of different formulations of BC on postharvest physiology and quality of plum cv. Angeleno

'Angeleno' plum with the fruit firmness of 38.4±4.0 N, TSS of 15.6±0.5 % and TA of 2.8±0.2 % were sourced from the Eastwind Farm on 9th March 2017. The same experimental procedure as mentioned in experiment 1 was applied to experiment 2, except the setting of 3 replications (90 fruit) per treatment due to the limitation of fruit availability.

4.2.1.3 Experiment 3: Effect of different formulations of BC on postharvest physiology and quality of plum cv. Tegan Blue

The commercially matured Tegan Blue plum with the fruit firmness of 37.1±4.0 N, TSS of 10.2±0.5 % and TA 1.81±0.2 % were sourced from the Eastwind Farm on 14th February 2017. Experiment 3 was carried out following the same experimental procedure of experiment 1. The fruit were kept in two different storage conditions: (1) under ambient at 20±1°C (85±5% RH) for 10 d and (2) under cold storage at 0±1°C (90±5% RH) for 40 d.

4.2.2 Determination of physiological parameters

4.2.2.1 Ethylene production

Ethylene production of all the tested cultivars was determined by following the methods detailed by Khan and Singh (2008) and Azzu (2016) with some modifications using Agilent 6890N Network Gas Chromatogram, (Agilent Technologies, Santa Clara, USA) and the ETD-300 ethylene detector (Sensor sense B.V, Nijmegen, The Netherlands). The ethylene production rate was expressed as μmol kg^{-1} h^{-1} for 'Fortune' and nmol kg^{-1} h^{-1} for 'Angeleno' and 'Tegan Blue' and the detailed procedure was mentioned in Chapter 3, section 3.5.1.

4.2.2.2 Respiration rate

The respiration rate of all three plum varieties was analysed by using the gas analyser (1450 series Gas Analyser, Servomex, United Kingdom) following the method described by Khan (2007). The determination of respiration rate is detailed in Chapter 3, section 3.5.2 and, respiration rate was mentioned as mmol CO_2 kg^{-1} h^{-1}.

4.2.2.3 Physiological weight loss

The initial and final fruit weight of each replication were recorded before and after the cold storage to calculate the percent physiological weight loss following the procedure detailed by Azzu (2016) as described in Chapter 3, section 3.5.3.

4.2.2.4 Firmness

The fruit firmness was determined by following the procedure outlined by Khan and Singh (2008) using the texture analyser (TPA Plus, AMETEK Lloyd Instruments, United Kingdom) with little modifications. The fruit firmness was expressed as N and the determination method was inclusively mentioned in Chapter 3, section 3.5.4.

4.2.3 Determination of quality parameters

4.2.3.1 SSC (%), TA (%) and SSC:TA

The soluble solids content was determined from the fresh juice using a portable digital refractometer (Atago-Palette PR 101, Atago Co., Itabashi-Ku, Tokyo, Japan) and expressed as a percentage. The percent titratable acidity was quantified from the fresh juice as malic acid equivalent by titrating against 0.1 N NaOH till the pale pink colour endpoint is reached in the presence of phenolphthalein as an indicator. The ratio of SSC:TA was calculated by dividing the value of SSC by the respective value of TA. The SSC, TA and SSC:TA were quantified by following the methods of Khan and Singh (2008) as described in Chapter 3, section 3.6.1.

4.2.3.2 Individual sugars and organic acids

The individual sugar and organic acid assays were undertaken by using a reversed-phase high-performance liquid chromatography (HPLC) (Binary HPLC Pump, Waters 1525, Milford Corp., MA, USA) by following the detailed procedure of Usenik et al.

(2008) with some modifications. The individual sugars and organic acids were expressed as g kg^{-1} fresh weight. The quantification procedure and the detailed description of the HPLC machine was inclusively outlined in Chapter 3, section 3.6.2.

4.2.3.3 Total phenols

The total phenolic assay was undertaken following the procedure inclusively described by Cantin et al. (2009) with modifications. The phenolic compounds from the fresh fruit pulp (20 g) were extracted according to the detailed procedure mentioned in section 3.6.3 and the phenolic assay was done by using a spectrophotometer (6405 UV/visible (190-1100 nm), Jenway, Dunmow, Essex, UK) at the wavelength of 750 nm and mentioned as gram gallic acid equivalents per kilogram (g GAE kg^{-1}) fresh pulp weight.

4.2.3.4 Ascorbic acid

The ascorbic acid level in the fruit pulp was quantified by the methods of Vithana (2017) by using a spectrophotometer (6405 UV/visible (190-1100 nm), Jenway, Dunmow, Essex, UK) at the wavelength of 760 nm. The detailed procedure of ascorbic acid assessment was mentioned in Section 3.6.4 and expressed as g kg^{-1} fresh weight.

4.2.3.5 Total antioxidant capacity

The total antioxidant capacity of the fruit pulp was quantified by the DPPH (free radical scavenging capacity) method as previously detailed by Vithana (2017) using a spectrophotometer (6405 UV/visible (190-1100 nm), Jenway, Dunmow, Essex, UK) at the wavelength of 515 nm. The total antioxidant was assessed by the detailed procedure mentioned in Section 3.6.6 and expressed as mmol Trolox equivalent antioxidant activity per kg (mmol TEAC kg^{-1}).

4.2.3.6 Total anthocyanin

The total anthocyanin content in the peel was quantified following the method described by Whales and Singh (2007) as mentioned in section 3.6.5. The assessment of total anthocyanin content was done by using a spectrophotometer (6405 UV/visible (190-1100 nm), Jenway, Dunmow, Essex, UK) at the wavelength of 530 nm and expressed as g kg^{-1} cyanidin-3-glucoside of fresh peel weight.

4.2.4 Statistical analysis

The results obtained were analysed by applying one-way or two-way analysis of variance using the statistical Genstat (13th edition) software, VSN International Ltd., UK. The treatment effects were compared by calculating the least significant differences (LSD) at ($P \leq 0.05$). Duncan's multiple range test was used for multiple comparisons of treatment means and the results were presented as means ± standard error of means. The physical and biochemical quality parameters, except ethylene production and respiration rate which were subjected to two-way ANOVA, for the respective plum cultivars and storage periods were analysed individually using one-way ANOVA.

4.3 Results

4.3.1 Ethylene production

BC formulations significantly suppressed ethylene production and delayed the climacteric peak onsets of all the tested Japanese plums after 25 and 40 d cold storage (Fig. 4.1).

Ethylene climacteric peaks of 'Fortune' plum treated with BC formulations, except for distilled water alone, were exhibited one day later (on 7th day of ripening period) than the untreated plum in both 25 and 40 d cold storage (Fig. 4.1 A and B). Control fruit showed the maximum ethylene production (56.3 and 35.1 µmol kg^{-1} h^{-1}, respectively), while the fruit treated with BC fumigation exhibited the lowest level (25.2 and 17.3 µmol kg^{-1} h^{-1}, respectively) after 25 and 40 d cold storage. Ethylene production of the fruit treated with BC aqueous solutions containing adjuvants Tween® 20, ethanol and β-cyclodextrin were not significantly different from BC fumigation after 25 and 40 d cold storage. The BC solution containing only distilled water significantly suppressed ethylene production of 'Fortune' plum to 1.5 fold compared to control fruit after 25 d cold storage but retained almost the same ethylene production level of control fruit after 40 d cold storage.

Ethylene peaks of 'Angeleno' plum treated with BC formulations, except BC solutions containing only distilled water and β-cyclodextrin in 25 d cold storage, were not exhibited till the end of ripening period and ethylene productions were significantly suppressed irrespective of BC formulations (Fig. 4.1 C and D). The climacteric peaks

of untreated ‘Angeleno’ plum were observed on 13th (24.1 nmol kg^{-1} h^{-1}) and 9th day (7.3 nmol kg^{-1} h^{-1}) after 25 and 40 d cold storage, respectively. The ethylene peaks of ‘Angeleno’ plum treated with BC solutions containing distilled water only and β-cyclodextrin were also exhibited on 13th day as the same peak-day in control after 25 d cold storage, however, the ethylene concentrations were significantly reduced to 1.3 fold and 2.5 fold, respectively.

Ethylene peaks of ‘Tegan Blue’ plum treated with BC, regardless of formulations, were revealed on 7th day which was one day later than the untreated fruit under ambient condition (Fig. 4.1 E). The fruit exposed to BC fumigation and BC solution containing ethanol exhibited the ethylene peaks 3 days later than the untreated fruit on 6th day after 40 d cold storage while the other formulations of BC treated fruit showed the peak one day later than control on 4th day of ripening period (Fig. 4.1 F). The control fruit revealed the highest climacteric ethylene concentrations (206.8 and 187.6 nmol kg^{-1} h^{-1}, respectively) after 25 and 40 d cold storage, whilst BC-fumigated fruit exhibited the lowest ethylene concentration (63.9 and 86.3 nmol kg^{-1} h^{-1}, respectively), followed by the fruit treated with BC solution containing ethanol (80.7 and 108.9 nmol kg^{-1} h^{-1}, respectively) (Fig. 4.1 E and F). The climacteric ethylene concentrations of the fruit treated with BC solution containing Tween® 20 (1.6 and 1.5 fold), β-cyclodextrin (1.28 and 1.27 fold) and only distilled water (1.19 and 1.18 fold) were lower to compared to control fruit after 25 and 40 d cold storage, respectively (Fig. 4.1 E and F).

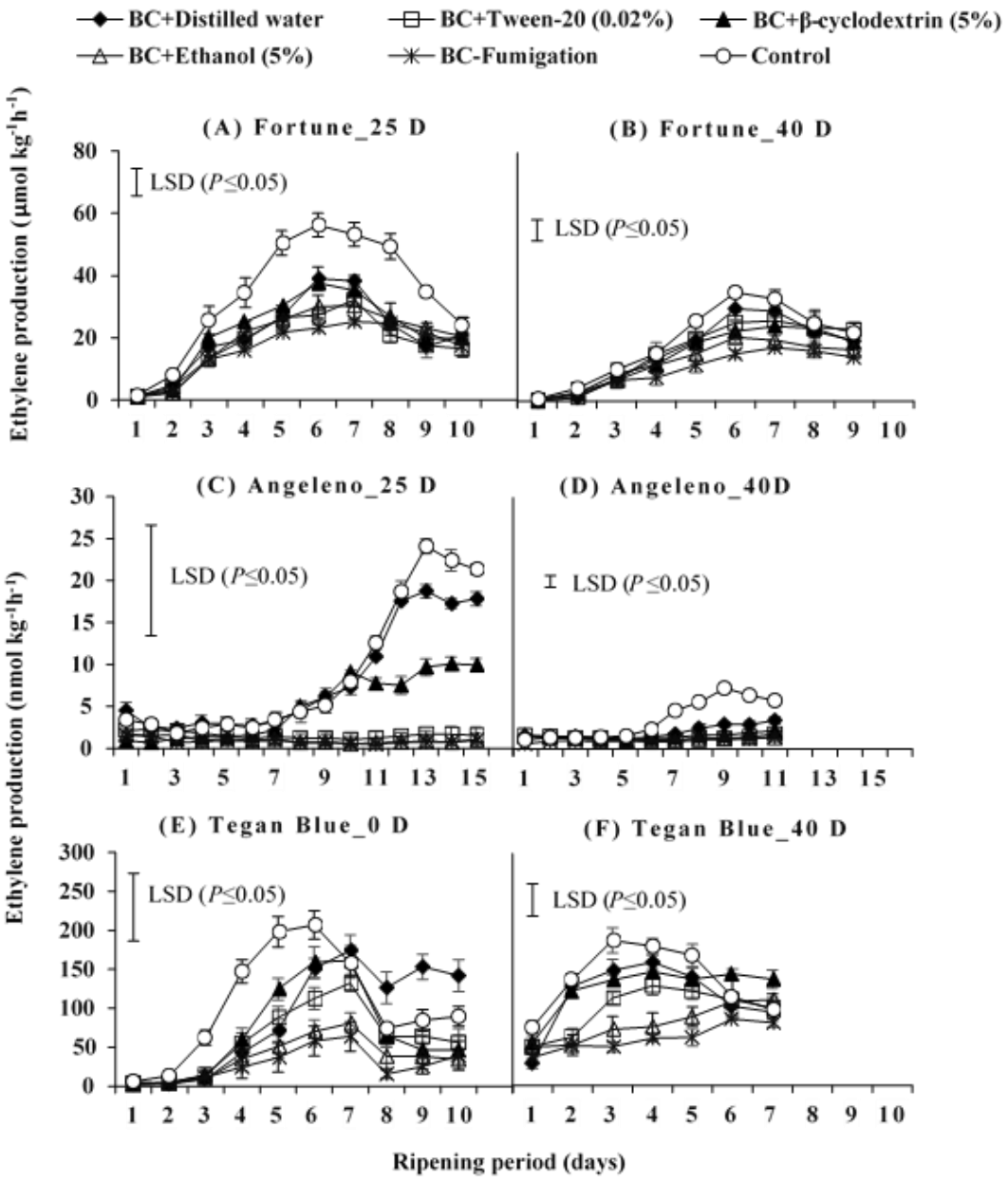


Fig. 4. 1 Effect of different aqueous solutions and fumigation treatments of BC on ethylene production of 'Fortune' (A and B) and 'Angeleno' (B and C) plum stored for 25 and 40 d, respectively and 'Tegan Blue' (E and F) plum stored for 0 and 40 d at 1°C. LSD bars represent treatments (tr) x days after storage (d). Vertical bars describe SE of means of three replicates and are not visible when the values are too small. LSD values for ethylene production of Angeleno (25 D): treatments (tr)=3.4, d after storage (d)=5.4 and their interaction (tr x d) =ns, Angeleno (40 D): tr =0.43, d =0.58, tr x d =1.4, Fortune (25 D): tr =2.78, d =3.6, tr x d =8.8, Fortune (40 D): tr =2.27, d =2.78, tr x d =ns. Tegan Blue (0 D): tr =27.5, d =35.5, tr x d =ns, Tegan Blue (40 D): tr =15.9, d =17.2, tr x d =42.1.

4.3.2 Respiration rate

Respiration rate and the peak of the tested cultivars responded to BC formulations differently, however, in general, they were not significantly affected.

The respiration peaks of 'Fortune' plum treated with BC formulations, except BC solution containing β-cyclodextrin which exhibited the peak onset two days later than the untreated fruit after 25 d storage, were not exhibited and their respiration rates were observed to be steady throughout the ripening period after 25 and 40 d storage (Fig. 4.2 A and B). The climacteric respiration peak of untreated 'Fortune' plum (1.77 mmol CO_2 kg^{-1} h^{-1}) was exhibited on 7^{th} day after 25 d cold storage, while no clear peak was observed after 40 d cold storage.

Neither 'Angeleno' plum treated with BC formulations nor control exhibited the climacteric respiration peak throughout ripening periods after 25 d cold storage (Fig. 4.2 A). When averaged over ripening period, the respiration rates of 'Angeleno' plum exposed to fumigation and BC solution containing ethanol were significantly lower (1.2 and 1.1 fold, respectively) compared to control fruit. The respiration rates of 'Angeleno' plum treated with BC solutions containing Tween® 20 and β-cyclodextrin were not significantly different from that of control whilst in contrast BC solution containing only distilled water exhibited significantly higher respiration rate as compared to control after 25 d cold storage (Fig. 4.2 A). After 40 d cold storage, 'Angeleno' plum treated with BC formulations revealed suppressed respiration rate, but not significant, starting from 5^{th} day of ripening period as compared to control fruit which exhibited the climacteric peak (1.6 µmol CO_2 kg^{-1} h^{-1}) on 5^{th} day of ripening period (Fig. 4.2 C).

The respiration rate and peak onset of 'Tegan Blue' plum were not affected by BC formulations under ambient condition (0 d storage) and the respiration rate was found to be increased after 5 d of ripening period regardless of the treatments (Fig. 4.2 C). When averaged over ripening period, the respiration rate of 'Tegan Blue' plum stored for 40 d at 0±1°C was significantly suppressed by BC formulations, whilst the peak was not observed till the end of ripening period in both treated and untreated fruit (Fig. 4.2 D). The significantly lowest average respiration rate was recorded in 'Tegan Blue' plum treated with BC fumigation, which was 1.1 fold lower than the respiration rate of control.

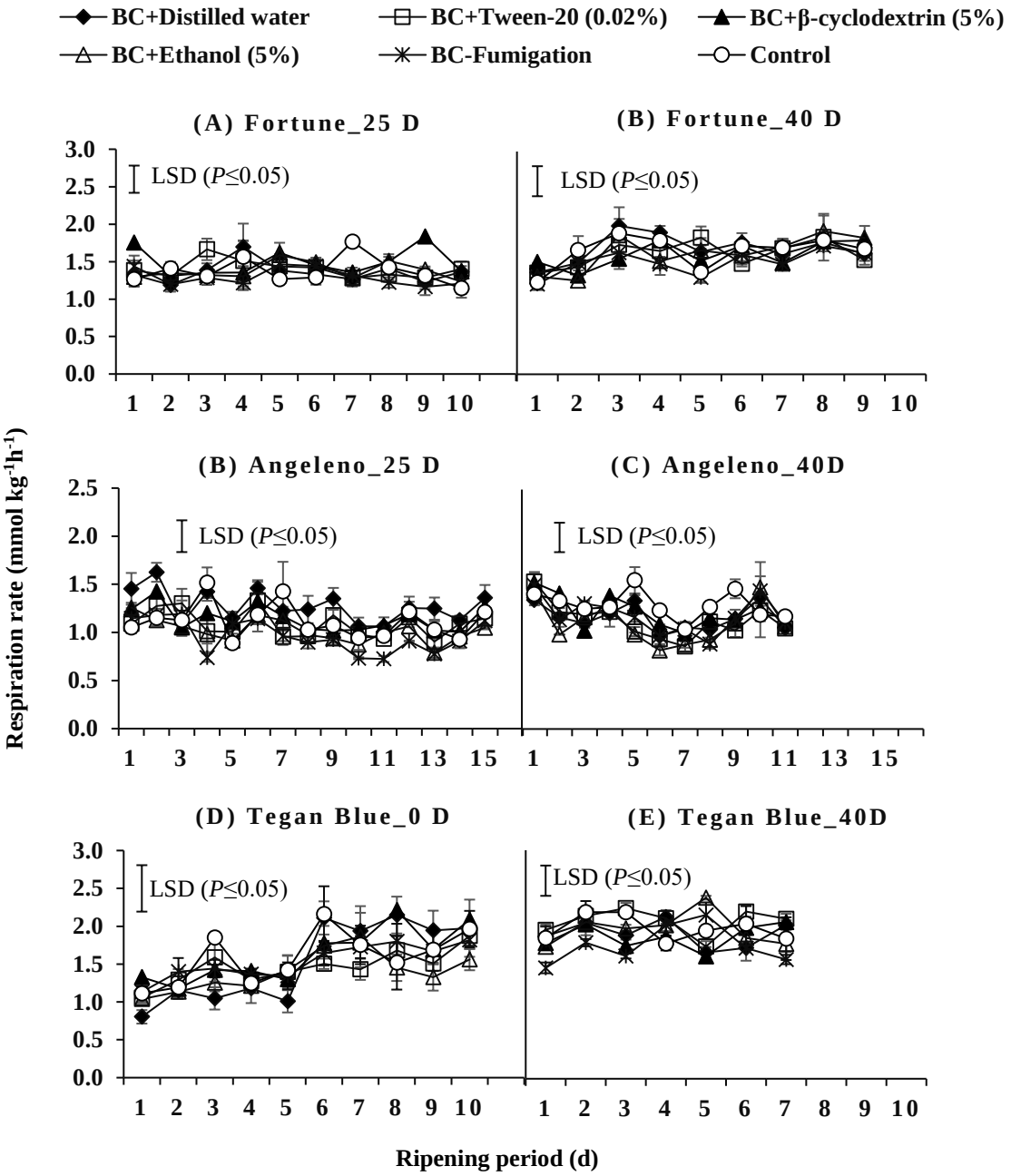


Fig. 4. 2 Effect of different aqueous solutions and fumigation treatments of BC on respiration rate of 'Fortune' (A and B) and 'Angeleno' (B and C) plum stored for 25 and 40 d, respectively and 'Tegan Blue' (E and F) plum stored for 0 and 40 d at 1°C. LSD bars represent treatments (tr) x days after storage (d).Vertical bars describe SE of means of three replicates and are not visible when the values are too small. LSD values for respiration rate of Angeleno (25 D): treatments (tr)=0.09, d after storage (d)=0.14 and their interaction (tr x d) =ns, Angeleno (40 D): tr =0.09, d =0.13, tr x d =ns, Fortune (25 D): tr =ns, d =ns, tr x d =ns, Fortune (40 D): tr =ns, d =0.17, tr x d =ns. Tegan Blue (0 D): tr =ns, d =0.25, tr x d =ns, Tegan Blue (40 D): tr =0.15, d =0.16, tr x d =0.40.

4.3.3 Physiological weight loss

Physiological weight losses of all the plum cultivars were significantly reduced by BC fumigation, BC solutions with ethanol and with Tween® 20 after 25 and 40 d. 'Fortune' plum treated with BC formulations, except BC solution with distilled water, significantly lowered weight loss as compared to control after 25 d cold storage (Fig. 4.3 A). Weight loss of the fruit treated with BC fumigation and BC solution containing Tween® 20 were recorded to be the lowest (2.0% each), followed by BC solutions containing ethanol and β-cyclodextrin (2.1 and 2.2%, respectively). There was no significant difference in the weight loss of the treated and untreated fruit after 40 d cold storage, nonetheless, the lowest percent weight loss was found in the BC fumigated fruit which was 1.7 fold lower than control (Fig. 4.3 B). 'Angeleno' plum treated with BC fumigation, BC solutions containing ethanol and Tween® 20 exhibited significantly less (3.4, 3.4 and 1.6 fold, respectively) weight loss than control, whilst the rest of BC formulations retained significantly the same weight loss as compared to control after 25 d cold storage (Fig. 4.4 A). After 40 d cold storage, the percent weight losses of the fruit treated with BC fumigation, BC solutions containing ethanol, Tween® 20 and β-cyclodextrin were significantly reduced (2.3, 2.2, 1.8 and 1.2 fold, respectively) as compared to untreated fruit (Fig. 4.4 B). 'Tegan Blue' plum treated with BC formulations, except BC solution containing only distilled water, significantly retained lower physiological weight after 40 d cold storage. The fruit exposed to BC fumigation, BC solutions containing ethanol, Tween® 20 and β-cyclodextrin exhibited significantly less (2.5, 2.0, 1.7 and 1.3 fold, respectively) weight loss as compared to untreated ones after 40 d cold storage (Fig. 4.5 A).

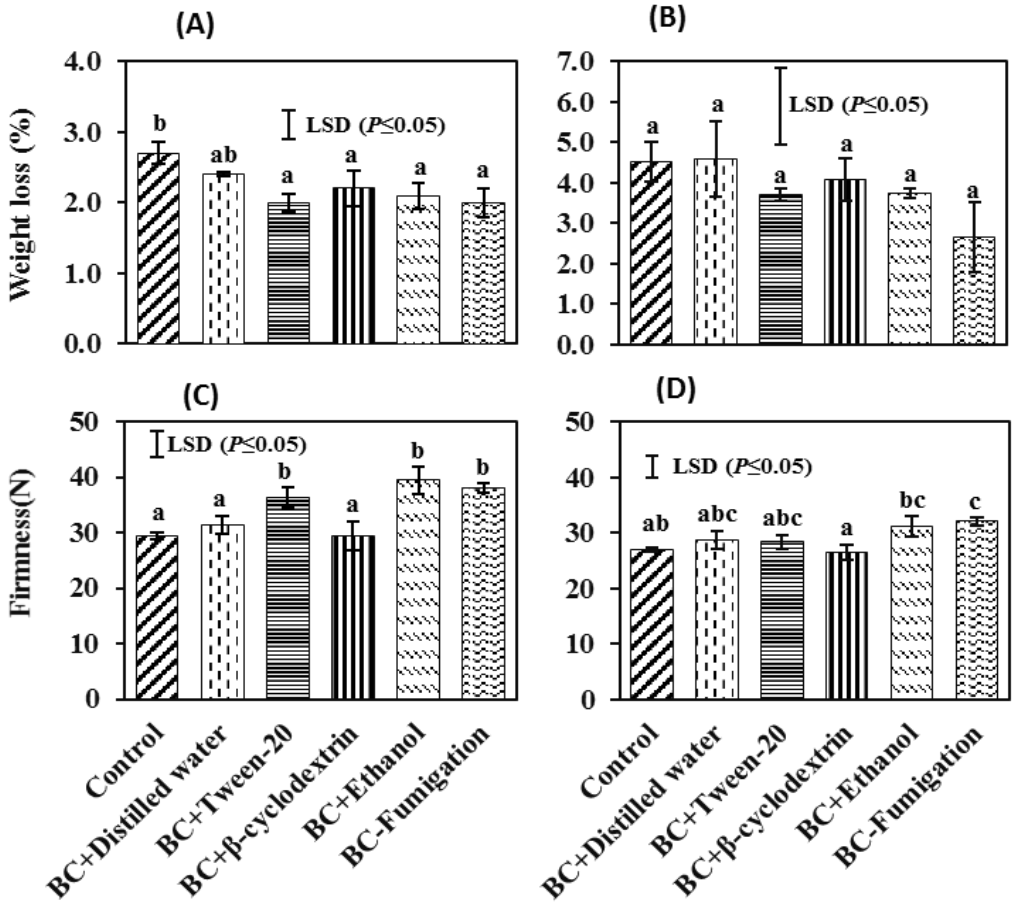


Fig. 4. 3 Effect of different BC aqueous solutions and fumigation treatments on the weight loss and firmness of 'Fortune' plum stored for 25 d (A and C) and 40 d (B and D), respectively at 1°C. Vertical bars describe SE of means of three replicates and are not visible when the values are too small. The treatments with the same letter are not significantly different from each other.

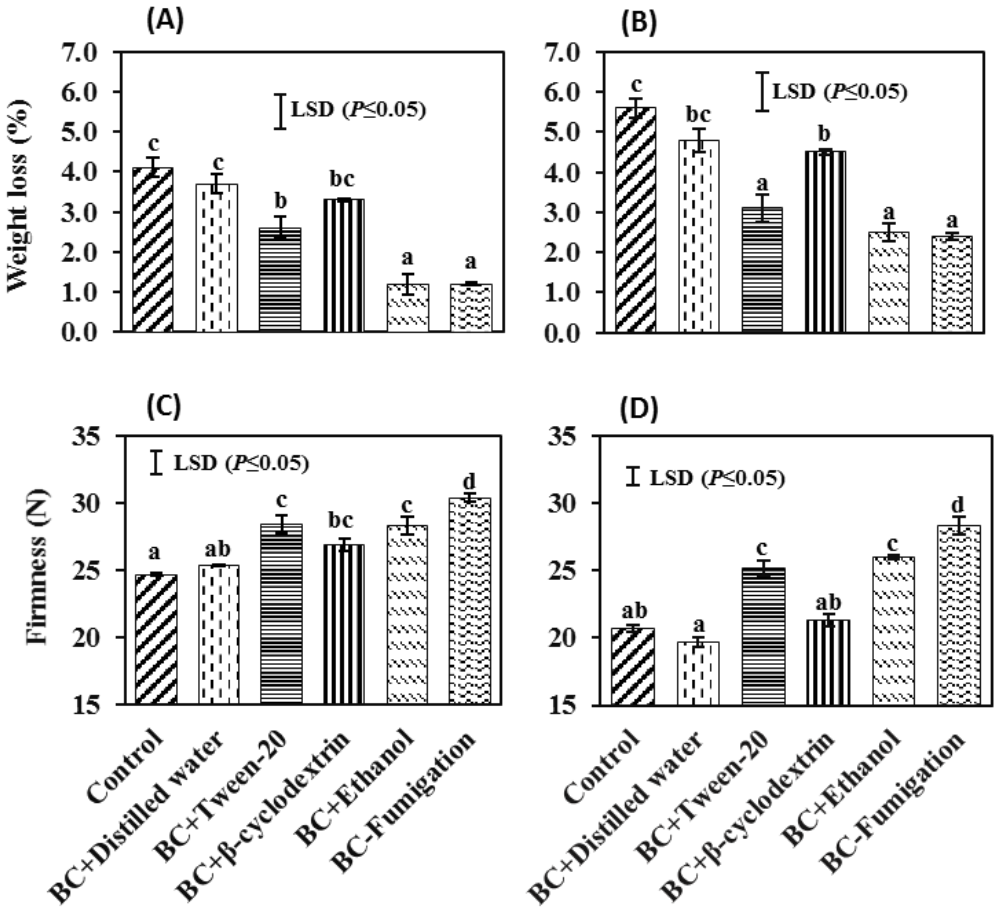


Fig. 4. 4 Effect of different BC aqueous solutions and fumigation treatments on the weight loss and firmness of ‘Angeleno’ plum stored for 25 d (A and C) and 40 d (B and D), respectively at 1°C. Vertical bars describe SE of means of three replicates and are not visible when the values are too small. The treatments with the same letter are not significantly different from each other.

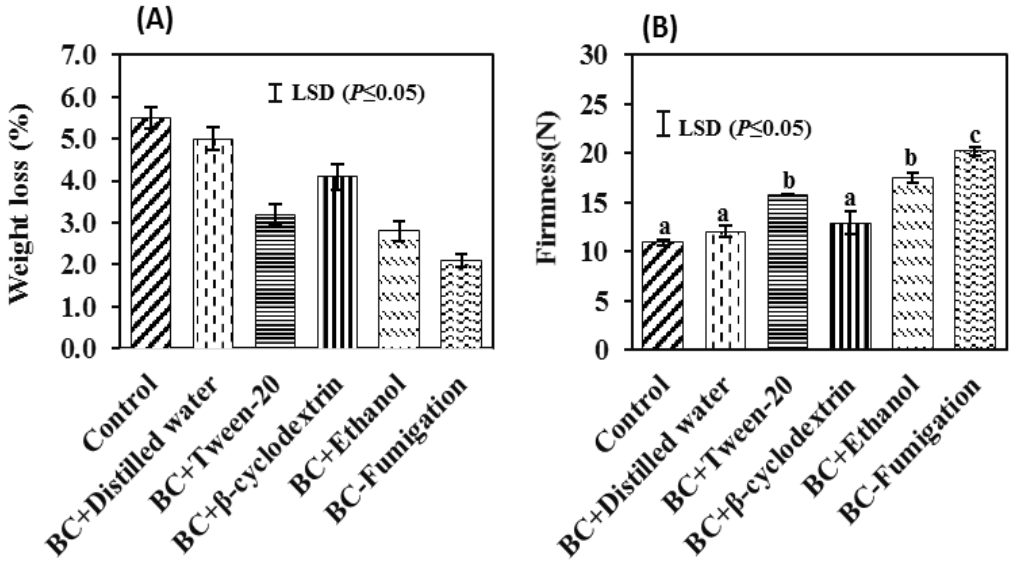


Fig. 4. 5 Effect of different BC aqueous solutions and fumigation treatments on the weight loss (A) and firmness (B) of 'Tegan Blue' plum stored for 40 d at 1°C. Vertical bars describe SE of means of three replicates and are not visible when the values are too small. The treatments with the same letter are not significantly different from each other.

4.3.4 Firmness

BC formulations maintained higher fruit firmness of all the plum cultivars after 25 and 40 d cold storage. After 25 d cold storage, 'Fortune' plum exposed to BC fumigation, BC-ethanol and BC-Tween 20 solutions exhibited significantly higher (1.3, 1.3 and 1.2 fold, respectively) fruit firmness compared to control, whilst fruit firmness of the rest of formulations were almost the same as that of control (Fig. 4.3 C). After 40 d cold storage, the firmness of the fruit treated with BC fumigation was observed to be the highest and, which was significantly 1.2 fold higher as compared to untreated fruit. The fruit treated with spray solutions of BC retained significantly the same firmness as compared to untreated fruit (Fig. 4.3 D). 'Angeleno' plum treated with BC fumigation, BC solutions containing ethanol, Tween® 20, and β-cyclodextrin significantly retained higher fruit firmness up to 1.2 fold as compared to control after 25 d cold storage (Fig. 4.4 C). Similarly, the fruit exposed to fumigation, BC solutions containing ethanol, and Tween® 20 showed significantly higher (1.4, 1.3 and 1.2 fold, respectively) fruit firmness as compared to control, while the fruit treated with BC

solutions containing only distilled water, and β-cyclodextrin retained the same firmness as control after 40 d cold storage (Fig. 4.4 D). Similarly, 'Tegan Blue' plum exposed to BC fumigation and BC solutions containing ethanol, and Tween 20 revealed significantly higher firmness up to 1.9 fold as compared to control. The firmness of the fruit sprayed with BC solutions containing distilled water, and β-cyclodextrin were significantly the same, but comparatively lower (1.1 and 1.2 fold, respectively) as compared to untreated fruit after 40 d storage (Fig. 4.5 B).

4.3.5 SSC, TA and SSC:TA

'Fortune' plum treated with BC formulations, except BC solution containing distilled water alone, retained lower level of SSC values (1.1 fold each) as compared to untreated fruit after 25 d cold storage, whilst in contrast, SSC levels of treated and untreated fruit were significantly the same after 40 d cold storage (Table 4.1). The SSC levels of 'Angeleno' plum sprayed with BC solutions containing Tween® 20, or ethanol were observed to be significantly the lowest, followed by BC fumigation, which were up to 1.2 fold lower as compared to control fruit after 25 d cold storage. Similarly, SSC levels of 'Angeleno' plum treated with BC formulations, except distilled water, were significantly lowered as compared to untreated fruit after 40 d cold storage. SSC of 'Tegan Blue' plum treated with BC formulations, irrespective of the adjuvant applied, were significantly lower as compared to control after 40 d cold storage. Similarly, the levels of SSC:TA ratio for 'Fortune', 'Angeleno' and 'Tegan Blue' plum treated with BC formulations, except BC solution containing distilled water, were significantly lower as compared to control after the 25 and 40 d storage periods (Table 4.1).

In contrast, levels of TA in 'Fortune', 'Angeleno' and 'Tegan Blue' plum treated with BC formulations were relatively higher as compared to control after 25 and 40 d cold storages (Table 4.1). The level of TA in 'Fortune' plum treated with BC solution containing ethanol was significantly higher (1.1 fold, each) as compared to control fruit, whilst TA of the fruit treated with the rest of formulations were not different from control after 25 and 40 d cold storages. Similarly, the level of TA in 'Angeleno' and 'Tegan Blue' plums exposed to BC fumigation and BC solutions containing Tween® 20, ethanol or, β-cyclodextrin were recorded to be significantly higher up to 1.4 fold respectively as compared to control after 25 and 40 d cold storages (Table 4.1).

Table 4. 1 Effect of different formulations of ethylene antagonist BC on SSC, TA and SSC:TA ratio of Fortune, Angeleno and Tegan Blue plums stored for 25 d and 40 d at cold storage (1 °C)

	Treatment	SSC (%)		TA (%)		SSC:TA	
		25 d	40 d	25 d	40 d	25 d	40 d
Fortune	Control	11.3±0.2b	10.9±0.2	1.5±0.0ab	1.3±0.02ab	7.5±0.11b	8.3±0.20ab
	BC+Distilled water	10.8±0.1ab	11.1±0.1	1.5±0.1 a	1.3±0.02a	7.3±0.16b	8.9±0.18b
	BC+Tween® 20	10.3±0.4a	10.7±0.2	1.6±0.1bc	1.3±0.04ab	6.5±0.15a	8.0±0.16ab
	BC+β-cyclodextrin	10.3±0.1a	10.8±0.1	1.5±0.0abc	1.2±0.02a	6.7±0.21a	8.8±0.22b
	BC+Ethanol	10.2±0.3a	10.7±0.2	1.7±0.0c	1.4±0.07b	6.2±0.09a	7.5±0.41a
	BC-Fumigation	10.3±0.2a	10.3±0.3	1.6±0.0bc	1.3±0.02ab	6.4±0.14a	7.7±0.30a
	LSD ($P \leq 0.05$)	0.6*	ns	0.11*	0.113*	0.51**	0.82*
Angeleno	Control	14.1±0.1d	16.13±0.1e	0.72±0.02a	0.67±0.03a	19.7±0.4c	24.2±1.0c
	BC+Distilled water	13.4±0.1cd	15.93±0.1de	0.74±0.03a	0.76±0.05ab	18.3±0.9c	21.2±1.3bc
	BC+Tween® 20	11.5±0.5a	13.97±0.2a	1.01±0.00c	0.92±0.07c	11.4±0.5a	15.5±1.2a
	BC+β-cyclodextrin	13.3±0.5cd	15.50±0.2cd	0.87±0.03b	0.83±0.02bc	15.3±0.3b	18.8±0.6ab
	BC+Ethanol	12.1±0.5ab	14.97±0.1bc	0.96±0.02c	0.87±0.03bc	12.6±0.6a	17.3±0.7a
	BC-Fumigation	12.7±0.2bc	14.83±0.2b	1.03±0.02c	0.83±0.02bc	12.3±0.2a	18.0±0.6ab
	LSD ($P \leq 0.05$)	1.01*	0.542**	0.08**	0.14*	2.05**	3.123*
Tegan Blue	Control		11.8±0.1c		1.1±0.03a		11.0±0.2e
	BC+Distilled water		9.9±0.4b		1.1±0.03ab		8.7±0.6d
	BC+Tween® 20		9.6±0.5b		1.3±0.03b		7.6±0.3c
	BC+β-cyclodextrin		10.2±0.3b		1.2±0.03ab		8.4±0.1d
	BC+Ethanol		7.4±0.2a		1.3±0.02b		5.7±0.1b
	BC-Fumigation		6.5±0.1a		1.5±0.08c		4.4±0.3a
	LSD ($P \leq 0.05$)		1.1**		0.16*		0.86**

n = 3 replicates (12 fruit per replication), ± 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 the same letter within the columns are not significantly different. The mean values of Fortune, Angeleno and Tegan Blue are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

4.3.6 Individual sugars

Glucose and fructose levels of ‘Fortune’, sucrose and sorbitol levels of ‘Angeleno’ and sucrose level of ‘Tegan Blue’ plum were significantly suppressed by BC formulations after 25 and 40 d storages (Table 4.2). Glucose levels of ‘Fortune’ plum treated with BC fumigation, BC solutions containing ethanol or, Tween 20 were significantly lowered (55, 42.6 and 34.8 % respectively), whilst the rest of formulations were not significantly different as compared to control after 25 d cold storage. In contrast, glucose levels of ‘Fortune’ stored for 40 d, ‘Angeleno’ and ‘Tegan Blue’ stored for 25 and 40 d were not significantly affected by BC formulations (Table 4.2).

The levels of fructose in ‘Fortune’, ‘Angeleno’ and ‘Tegan Blue’ plum treated with BC formulations, except BC solution containing distilled water only, remained the same as in control fruit after 25 and 40 d storages (Table 4.2). The levels of sucrose in ‘Fortune’ plum treated with BC formulations were maintained significantly the same as in control after 25 and 40 d cold storages. The levels of sucrose in ‘Angeleno’ plum treated with BC fumigation, BC solutions containing ethanol and, β-cyclodextrin were significantly lowered (1.4, 1.2 and 1.2 fold, respectively) as compared to control after 25 d cold storage. Similarly, after 40 d cold storage, sucrose levels of ‘Angeleno’ treated with BC fumigation, BC solutions containing ethanol, Tween® 20 and, β-cyclodextrin were significantly reduced up to 1.1 fold as compared to control (Table 4.2). In contrast, the levels of sucrose in ‘Tegan Blue’ plum treated with BC solutions containing ethanol and, β-cyclodextrin were found to be significantly higher (2.0 and 1.7 fold, respectively) as compared to control after 40 d cold storage (Table 4.2). The levels of sorbitol in ‘Fortune’ and ‘Tegan Blue’ plum treated with BC formulations remained similar to those in control fruit stored for 25 and 40 d. Sorbitol levels in ‘Angeleno’ plum exposed to BC fumigation, BC solution containing ethanol, Tween® 20 and, β-cyclodextrin were significantly lowered (1.3, 1.2, 1.2 and 1.1 fold, respectively) as compared to control after 25 d cold storage. Similarly, sorbitol levels in the fruit treated with BC fumigation and BC solution containing Tween® 20 were significantly reduced (1.2 fold each), while that of the fruit treated with rest of BC formulations were not significantly different as compared to control after 40 d cold storage (Table 4.2).

Table 4. 2 Effect of different formulations of ethylene antagonist BC on the individual sugars of Fortune, Angeleno and Tegan Blue plum stored for 25 d and 40 d at cold storage (1°C)

	Treatment	Glucose (g kg^{-1})		Fructose (g kg^{-1})		Sucrose (g kg^{-1})		Sorbitol (g kg^{-1})	
		25 d	40 d	25 d	40 d	25 d	40 d	25 d	40 d
Fortune	Control	48.6±1.3c	64.2±2.8	35.3±2.1abc	53.7±2.2	49.3±5.0	28.1±3.3	21.7±1.8	21.1±0.9
	BC+Distilled water	39.2±3.4bc	74.5±2.2	45.0±0.9d	59.0±2.1	36.5±3.7	30.2±4.3	22.4±1.3	21.2±1.0
	BC+Tween® 20	31.7±2.9ab	57.4±3.9	32.3±1.7ab	50.4±2.3	25.6±4.3	27.5±5.6	15.4±2.0	18.8±0.6
	BC+β-cyclodextrin	45.0±4.9c	62.2±2.9	43.3±2.1cd	57.4±2.2	38.0±3.8	39.2±1.0	24.3±0.7	22.9±1.1
	BC+Ethanol	27.9±3.3ab	58.4±6.2	40.0±1.9bcd	54.4±1.9	39.4±4.8	37.2±8.3	22.1±1.0	23.4±2.9
	BC-Fumigation	21.8±3.6a	54.9±5.5	29.6±4.5a	54.1±2.8	40.7±6.9	38.7±5.5	20.4±3.3	23.7±2.1
	LSD ($P \leq 0.05$)	10.79**	ns	7.836*	ns	ns	ns	ns	ns
Angeleno	Control	170.2±3.4	184.0±3.1	254.7±11.3	263.9±5.2	29.5±1.2cd	34.6±0.4b	72.8±2.5c	67.8±2.4b
	BC+Distilled water	170.0±4.3	187.3±1.7	254.0±6.2	269.5±2.5	30.6±0.3d	33.0±0.3ab	71.1±2.0bc	69.3±1.9b
	BC+Tween® 20	159.0±1.3	178.7±1.0	237.5±2.5	256.9±2.5	26.8±0.3bc	32.6±0.4a	62.9±0.9ab	56.4±2.5a
	BC+β-cyclodextrin	173.4±1.9	186.5±1.5	261.7±2.7	265.8±2.6	24.8±1.4b	32.2±0.5a	63.9±2.2ab	70.7±1.6b
	BC+Ethanol	162.9±3.9	186.6±3.3	242.6±7.5	268.3±5.4	25.4±0.8b	31.5±0.3a	61.4±1.9a	74.9±2.7b
	BC-Fumigation	167.6±1.5	184.7±2.7	233.1±3.4	253.4±3.5	20.5±1.2a	32.3±0.7a	57.5±1.7a	56.2±2.6a
	LSD ($P \leq 0.05$)	ns	ns	ns	ns	3.48**	1.79*	7.99*	8.95*
Tegan Blue	Control		57.3±1.7		46.9±0.1		16.4±0.9a		24.6±1.3
	BC+Distilled water		56.9±7.8		45.9±0.0		21.4±2.4ab		24.2±4.1
	BC+Tween® 20		51.5±1.6		43.8±0.1		21.7±2.1ab		20.2±2.7
	BC+β-cyclodextrin		54.5±5.2		47.0±0.0		28.6±0.9bc		25.9±1.8
	BC+Ethanol		48.6±2.2		40.3±0.1		32.3±4.6c		26.9±2.0
	BC-Fumigation		37.3±0.9		43.3±0.03		22.9±1.7abc		20.9±1.9
	LSD ($P \leq 0.05$)		ns		ns		9.14*		ns

n = 3 replicates (12 fruit per replication), ± 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 the same letter within the columns are not significantly different. The mean values of Fortune, Angeleno and Tegan Blue are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

4.3.7 Individual organic acids

In general, the levels of individual organic acid, except citric and fumaric acids in 25 d cold storage, were not significantly different between the treated and untreated plums, however, organic acids in the fruit treated with BC formulations were comparatively lower than control after 25 and 40 d cold storages (Table 4.3). In all three plum cultivars, malic found to be predominant among the quantified organic acids in both storage periods and fumaric acid was not detected in 'Tegan Blue' plum stored for 40 d under cold storage (Table 4.3).

Citric acid levels of 'Fortune' plums treated with BC fumigation, BC solutions containing ethanol and, β-cyclodextrin were significantly higher (≈ 29, 26 and 25 %, respectively) as compared to control after 25 d storage. Similarly, the fumaric acid content of 'Fortune' plum treated with BC solution containing ethanol was significantly higher (≈ 17%) than control after 25 d cold storage. However, in contrast, the levels of citric and fumaric acids in 'Fortune', 'Angeleno' and 'Tegan Blue' plum treated with BC formulations were retained the same as in control fruit after 40 d cold storage. The concentrations of malic and succinic acid of 'Fortune', 'Angeleno' and 'Tegan Blue' plum stored for 25 and 40 d remained the same as compared to control (Table 4.3).

4.3.8 Total phenols

Total phenolic content 'Fortune' plum treated with BC solution containing Tween® 20 was significantly higher (1.2 fold) as compared to that of control which was not significantly different from the other BC formulations after 25 d cold storage (Table 4.4). Total phenolic contents of 40 d cold-stored 'Fortune', 25 and 40 d cold-stored 'Angeleno' and 40 d cold-stored 'Tegan Blue' were not significantly affected by BC formulations (Table 4.4).

Table 4. 3 Effect of different formulations of ethylene antagonist BC on the individual organic acids of Fortune, Angeleno and Tegan Blue plum stored for 25 d and 40 d at cold storage (1°C)

	Treatment	Malic acid (g kg^{-1})		Citric acid (g kg^{-1})		Fumaric acid (g kg^{-1})		Succinic acid (g kg^{-1})	
		25 d	40 d	25 d	40 d	25 d	40 d	25 d	40 d
Fortune	Control	34.7±3.9a	34.5±2.6	0.39±0.03a	0.43±0.04	0.29±0.01a	0.26±0.02	3.6±0.4a	3.44±0.3
	BC+Distilled water	37.6±1.0ab	34.5±2.4	0.42±0.02ab	0.47±0.05	0.29±0.01a	0.23±0.01	4.1±0.1a	3.29±0.1
	BC+Tween® 20	43.0±0.8b	39.0±2.0	0.47±0.02abc	0.46±0.02	0.28±0.01a	0.25±0.00	4.3±0.1a	3.95±0.1
	BC+β-cyclodextrin	36.0±1.9a	35.3±1.2	0.52±0.04bc	0.46±0.01	0.27±0.01 a	0.25±0.01	4.3±0.3a	3.29±0.1
	BC+Ethanol	35.7±2.5a	36.6±1.8	0.53±0.04c	0.45±0.01	0.35±0.02b	0.24±0.00	3.7±0.4a	3.43±0.3
	BC-Fumigation	41.1±1.1ab	38.4±2.3	0.55±0.02c	0.43±0.02	0.28±0.01a	0.25±0.00	4.1±0.2a	3.39±0.1
	LSD ($P \leq 0.05$)	ns	ns	0.097*	ns	0.034**	ns	ns	ns
Angeleno	Control	28.9±2.7	27.0±1.04	0.58±0.02	0.74±0.12	0.206±0ab	0.25±0.02	4.9±0.1	5.39±0.2
	BC+Distilled water	29.5±1.3	28.9±1.00	0.57±0.07	0.61±0.03	0.197±0a	0.22±0.01	5.0±0.3	5.15±0.1
	BC+Tween® 20	30.3±0.5	30.2±0.20	0.70±0.01	0.64±0.01	0.211±0b	0.24±0.01	5.2±0.3	5.27±0.2
	BC+β-cyclodextrin	28.3±0.9	29.1±0.44	0.61±0.02	0.73±0.10	0.201±0a	0.24±0.01	5.1±0.1	5.31±0.1
	BC+Ethanol	29.9±1.2	29.6±1.68	0.71±0.01	0.62±0.04	0.198±0a	0.22±0.00	5.5±0.1	5.42±0.2
	BC-Fumigation	30.6±0.4	30.8±0.39	0.73±0.02	0.58±0.02	0.203±0ab	0.23±0.00	5.7±0.1	5.42±0.2
	LSD ($P \leq 0.05$)	ns	ns	ns	ns	0.008*	ns	ns	ns
Tegan Blue	Control		20.6±0.04		0.179±0		nd		0.91±0.08
	BC+Distilled water		20.9±0.25		0.184±0		nd		0.83±0.02
	BC+Tween® 20		20.8±0.13		0.185±0		nd		0.94±0.09
	BC+β-cyclodextrin		21.0±0.20		0.184±0		nd		0.88±0.02
	BC+Ethanol		20.8±0.06		0.180±0		nd		0.86±0.08
	BC-Fumigation		21.2±0.18		0.186±0		nd		0.77±0.03
	LSD ($P \leq 0.05$)		ns		ns				ns

n = 3 replicates (12 fruit per replication), ± 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 the same letter within the columns are not significantly different. The mean values of Fortune, Angeleno and Tegan Blue are independent from each other. nd = not detected. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

4.3.9 Ascorbic acid

'Fortune' plum treated with BC formulations retained a tendentially higher level of ascorbic acid contents up to 28.7 and 15.5 % as compared to control after 25 and 40 d cold storage, respectively (Table 4.7). The fruit treated with BC solution containing Tween® 20 indicated highest ascorbic acid content (1.4 fold higher than control) after 25 d cold storage, whilst the significantly highest ascorbic acid content was observed in BC fumigated fruit (1.2 fold higher than control) after 40 d cold storage. The levels of ascorbic acid in 'Angeleno' and 'Tegan Blue' plum treated with BC formulations retained significantly the same as in untreated fruit after 25 and 40 d cold storage (Table 4.4).

4.3.10 Total antioxidants and anthocyanin

Antioxidant capacity of 'Fortune', 'Angeleno' and 'Tegan Blue' plum treated with BC formulations remained significantly the same as in control after 25 and 40 d cold storages (Table 4.4). Anthocyanin contents of 'Fortune', 'Angeleno' and 'Tegan Blue' plum treated with BC formulations were tendentially lower than that of control after the 25 and 40 d storage periods (Table 4.4). The levels of anthocyanin in 'Fortune' plum exposed to BC fumigation and BC solution containing Tween® 20 were significantly lower (≈ 27.5 and 16 %, respectively) as compared to control after 25 d cold storage. The anthocyanin contents of the fruit treated with BC fumigation, BC solutions containing Tween® 20 and, ethanol were significantly lesser (25, 21 and 18%, respectively) as compared to control after 40 d cold storage. Similarly, 'Angeleno' plum treated with BC formulations showed significantly lower levels of anthocyanin up to 40% after 40-day cold storage. In the same way, the anthocyanin contents of 'Tegan Blue' plum treated with BC solution containing Tween® 20 and, ethanol and BC fumigation were significantly lower (26, 20 and 17 %, respectively) as compared to control after 40 d cold storage (Table 4.4).

Table 4. 4 Effect of different formulations of ethylene antagonist BC on the total phenols, ascorbic acid, antioxidant capacity and anthocyanin contents of Fortune, Angeleno and Tegan Blue plum stored for 25 d and 40 d at cold storage (1°C)

	Treatment	**Total phenols (g GAE kg^{-1})**		**Ascorbic acid (g kg^{-1})**		**Antioxidants capacity (mmol TEAC kg^{-1})**		**Anthocyanin (g kg^{-1})**	
		25 d	40 d	25 d	40 d	25 d	40 d	25 d	40 d
Fortune	Control	72.3±2.9	84.3±5.4	11.9±0.3a	16.3±0.3ab	18.0±1.0	17.2±1.7	36.4±1.9c	37.1±1.7b
	BC+Distilled water	74.9±2.2	85.9±5.9	13.8±0.8b	15.6±0.7a	20.6±1.3	16.9±0.4	35.4±2.0c	35.6±1.3b
	BC+Tween® 20	83.0±7.6	78.0±2.5	16.7±0.6d	17.8±0.6c	19.8±1.8	17.5±0.1	30.6±1.0ab	29.3±1.2a
	BC+β-cyclodextrin	68.6±2.3	78.7±8.8	15.5±1.1cd	16.1±0.7a	19.1±0.1	19.7±0.5	34.4±0.6bc	32.6±0.7ab
	BC+Ethanol	69.2±3.9	71.7±8.1	15.6±0.5cd	17.7±0.5bc	19.7±1.7	17.6±0.9	33.6±0.6bc	30.6±0.8a
	BC-Fumigation	67.5±5.8	70.3±2.8	14.9±0.8bc	19.3±0.6d	21.4±0.4	20.6±1.0	26.4±0.2a	27.8±0.9a
	LSD ($P \leq 0.05$)	ns	ns	1.54**	**	ns	ns	4.13*	4.51*
Angeleno	Control	86.3±4.6	87.8±3.5	11.1±0.8	12.3±1.07	17.6±1.6	22.2±3.6		13.4±0.5b
	BC+Distilled water	77.1±4.2	84.3±5.3	11.3±0.5	12.6±0.13	20.8±2.4	22.4±2.3		9.8±0.5a
	BC+Tween® 20	78.9±2.9	83.6±7.7	11.2±0.1	12.2±0.12	16.7±1.2	16.9±0.5		9.5±0.6 a
	BC+β-cyclodextrin	82.3±3.3	92.6±3.9	11.1±0.3	11.7±0.53	16.3±0.8	16.0±0.8		8.9±1.0a
	BC+Ethanol	72.5±5.7	78.7±2.9	11.2±0.6	12.3±0.18	18.1±0.9	21.2±0.8		9.9±1.0a
	BC-Fumigation	79.5±6.8	94.8±2.7	11.3±0.5	12.6±0.49	20.5±2.3	20.9±2.6		8.0±1.1a
	LSD ($P \leq 0.05$)	ns	ns	ns	ns	ns	ns		2.55*
Tegan Blue	Control		70.0±9.8		10.4±0.5		15.7±1.2		30.4±1.2c
	BC+Distilled water		76.2±2.9		10.0±0.2		16.1±3.3		36.4±3.3d
	BC+Tween® 20		71.5±11.2		11.2±0.2		14.9±1.4		22.5±1.4a
	BC+β-cyclodextrin		75.6±10.9		10.8±0.6		15.5±1.4		29.0±1.4bc
	BC+Ethanol		82.2±15.8		10.6±0.5		16.2±2.4		25.2±2.4ab
	BC-Fumigation		80.6±6.4		10.9±1.1		15.7±0.8		24.2±0.8a
	LSD ($P \leq 0.05$)		ns		ns		ns		4.50**

n = 3 replicates (12 fruit per replication), ± 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 the same letter within the columns are not significantly different. The mean values of Fortune, Angeleno and Tegan Blue are independent from each other.

4.4 Discussion

4.4.1 Ethylene production

In the present study, ethylene production of 'Angeleno' and 'Tegan Blue' plums were comparatively lower than 'Fortune' plum indicating the two distinct behaviours of climacteric and suppressed-climacteric nature as previously observed by Abdi et al. (1997). The similar suppressed-climacteric ethylene production patterns were reported in 'Shiro', 'Rubyred' (Abdi et al., 1998), 'Tegan Blue' (Jobling et al., 2003), 'Angeleno' (Singh and Khan, 2010; Minas et al., 2015) 'Gaixian' plums (Pan et al., 2016). In this study, ethylene production of all the tested plums declined with the increase in cold storage period. This is in line with the results of Argenta et al., (2003) where the ethylene production of 'Laetitia' plums stored for 20, 30, 40 and 50 d at 1°C were decreased up to 50% with the increase in cold storage periods. Ethylene production was significantly retarded, and the onset of the peak was delayed by BC formulations irrespective of the adjuvants applied. The retardation of ethylene production by BC formulations may be triggered by binding of ethylene antagonists to the copper cofactor in the ethylene receptors and, thereby blocking the action of ethylene and the cellular response as previously reported by Sisler and Serek (1997), Alonso and Stepanova (2004) and Sisler et al. (2006). A similar inhibitory effect of 1-MCP on climacteric peak onset and production of ethylene has been reported in different climacteric and suppressed-climacteric plum cultivars (Khan and Singh, 2007a; Ozkaya et al., 2010; Minas et al., 2015; Singh et al., 2018). The antagonistic effects were more pronounced in BC fumigation, BC solutions containing ethanol and, Tween® 20 as compared to other BC solutions. Similar to the observations for 1-MCP (Sisler, 2006), BC achieves the highest efficiency when it is applied as a fumigant. Ethanol may enhance the delivery of BC in aqueous solutions to the targeted ethylene receptors in the fruit tissue. It has been reported that ethanol increases the solubility of cyclopropenes (Grichko, 2006), reduces the barrier properties of fruit cuticle by disrupting the structure of lipid bilayers (Patra et al., 2006) and subsequently, promotes the penetration of active compounds through the fruit surface (Farag et al., 1992). The higher efficacy of BC solution in the presence of Tween® 20 may be due to the amphiphilic structure of non-ionic surfactant Tween® 20 which enhances the delivery of treatment action and the uptake of the active compound by the fruit. The hydrophilic part of the surfactant promotes the water solubility of the active compound while the

hydrophobic part increases the solubility of lipid layers in the cuticle enhancing the absorption of the active compound through the fruit tissues (Parr and Norman, 1965; Stock and Holloway, 1993; Castro et al., 2014). The role of surfactants to enhance the performance of agrochemicals have been reported by Farag et al. (1992) in cranberry, Roy et al. (1996) in apples and Sing et al. (2000) in mango.

4.4.2 Respiration rate

The respiration rates of all the plums were found to be steady throughout the ripening periods and no significant climacteric respiratory peak was observed in all the tested plum cultivars irrespective of the BC formulations treated. The peaks may have occurred during the cold storage. Lelievre et al. (1997) reported that the respiratory climacteric peak may be prior to, or significantly delayed after the climacteric ethylene rise, or might not occur during the fruit ripening. For example, the 'Rubyred' plums neither untreated nor treated with different concentrations of 1-MCP exhibited the respiratory peak even after 16 d of ripening period (Abdi et al., 1998). The BC formulations did not show any significant effect on the respiration rate of all the cultivars under cold storage period. Similarly, the respiration rate of 'Red Rosa' plum after 35 d cold storage at 0°C (Dong et al., 2001), of 'Almog' and 'Oded' peaches stored for 5 d at 0°C and 'April Glow' nectarine stored for 7 d at 20°C (Liguori et al., 2004), of 'Black Amber' plums which are harvested at the commercial ripe stage and stored at 0°C for up to 45 d (Ozkaya et al., 2010) were also not significantly affected by 1-MCP irrespective of the concentration applied.

4.4.3 Physiological weight loss and firmness

The physiological weight loss was significantly reduced, and the fruit firmness was maintained by the BC formulations regardless of the adjuvants applied in all the cultivars. The inhibition of ethylene action with BC formulations might have suppressed the activity of enzymes which are related to fruit softening. According to Khan and Singh (2007), the retention of fruit firmness in 'Tegan Blue' plum treated with 1-MCP was attained by suppressing the activity of softening enzymes such as pectin esterase, endo-1,4-β-D-glucanase, exo-polygalacturonase and endo-polygalacturonase but the response was concentration-dependent. Martinez-Romero et al. (2003) also found that 1-MCP can reduce the weight loss and maintain the fruit

firmness of climacteric ‘Santa Rosa’ and non-climacteric ‘Golden Japan’ plums stored at 1°C. Similarly, the weight loss reduction and firmness retention by 1-MCP was observed in ‘President’ plum (Valero et al., 2003). However this observation is not universal, the weight loss of ‘Black Amber’ plum which are harvested at the commercial ripe stage and stored at 0°C for up to 45 d were not affected by 1-MCP (Ozkaya et al., 2010).

4.4.4 SSC, TA and SSC:TA

The levels of SSC and SSC:TA of the fruit treated with BC were generally lowered, while the TA contents were higher as compared to control fruit irrespective of the adjuvants applied and the cultivars. This indicates that the ethylene antagonist reduced ethylene production and slowed down the subsequent ripening process (Martinez-Romero et al., 2007). Similarly, the SSC percent, TA percent and SSC:TA ratio of ‘Songold’ plum were significantly influenced by 1-MCP (Velardo-Micharet et al., 2017). The contrary responses of SSC and SSC:TA ratio to ethylene antagonists were reported in ‘Black Amber’ and ‘Red Lane’ plums (Minas et al., 2013) and in ‘Royal Zee’ plum (Dong et al., 2002), however, the TA percent in these plums were maintained higher similar to the results obtained. In the present study, the percent SSC was observed to increase while the TA percent was decreased with the extension of cold storage period as previously described by Ozkaya et al. (2010) in ‘Black Amber’ plum which are harvested at the commercial ripe stage and stored at 0°C for up to 45 d.

4.4.5 Individual sugars and organic acids

In all the plum cultivars, fructose and malic found to be the predominant sugars and organic acid although the concentrations were greatly diverse depending on the cultivars as reported by Singh and Singh (2008) and Singh et al. (2009) in ‘Black Amber’, ‘Amber Jewel’ and ‘Angeleno’ plums. There was little variation in levels of sugars and organic acids with the increase in cold storage period. In general, individual sugars and organic acids, except the glucose and fructose in ‘Fortune’ plums and sucrose in ‘Angeleno’ plums, were not significantly influenced by the BC formulations. The reduction in glucose, fructose and sucrose might have resulted from

the inhibition of ethylene production which regulates sugar metabolism during fruit ripening (Defilippi et al. 2004; Kim et al. 2015).

4.4.6 Total phenols, ascorbic acid, antioxidant activity and anthocyanins

The levels of total phenols, ascorbic acid, antioxidant capacity and anthocyanin contents were varied among the tested cultivars and their concentrations increased in 40-day cold storage. Similar variation in the levels of these compounds depending on the cultivars was reported by Gil et al. (2002) and Kim et al. (2003) in peaches, nectarines and plums. In 'Angeleno' and 'Tegan Blue' plums, the total phenols, ascorbic acid and antioxidant capacity were not affected by the BC formulations similar to the previous reports in 'Pedro Sato' guava (Bassetto et al., 2005), in 'Chokanan' mango (Pauziah et al., 2014) and in 'Greensleeves' apples (Defilippi et al. 2004) treated with 1-MCP. However, the ascorbic acid levels of 'Fortune' plum were increased by BC fumigation and BC solutions containing Tween® 20 in both cold storage periods. This might be due to the retardation of ripening caused by the ethylene antagonist as previously reported by Singh and Pal (2008) in 'Allahabad Safeda' guava treated with 1-MCP (300 and 699 nL L^{-1}). The anthocyanin concentrations were generally reduced by BC formulations irrespective of the adjuvants, but the responses were varied depending on the cultivars. This may be due to the relation between ethylene production and accumulation of anthocyanin during fruit ripening. The influence of ethylene on anthocyanin accumulations was reported by Costa et al. (2012) in 'Bluecrop' and 'Goldtraube' blueberries and by Farag et al. (1992) in cranberry.

4.5 Conclusion

BC formulations effectively retarded ethylene production and physiological weight loss and maintained the other tested postharvest qualities of plums. However, the response of physiological and quality parameters, except ethylene production, to BC solutions with β-cyclodextrin and BC solution with only distilled water were inconsistent. The presence of β-cyclodextrin and the distilled water in BC aqueous solutions might not have helped to increase the solubility of BC or the permeability of BC aqueous solutions through fruit cuticle, like ethanol and Tween® 20 did.

Therefore, the addition of either ethanol or Tween® 20 while preparing the BC aqueous formulations can assure the greater performance of BC in inhibiting ethylene production and in delaying ethylene-related physiology and quality changes during ripening of plum. Overall, application of the ethylene antagonist BC before the cold storage, either as fumigation or as spray solution, has great potential in preserving the postharvest quality of plums by suppressing ethylene production.

CHAPTER 5

Impact of 1*H*-cyclopropa[*b*]naphthalene applied as fumigation or spray solutions on postharvest physiology and quality parameters of Japanese plums

Summary

The role of 1*H*-cyclopropa[*b*]naphthalene (NC) as a potential ethylene antagonist, either as fumigation or as spray solutions, on postharvest physiology and quality of two Japanese plums 'Tegan Blue' and 'Angeleno' was investigated by conducting two individual experiments. In both experiments, the freshly harvested plums without any defects were subjected to five different formulations of NC: NC fumigation (1 µM), NC solution (2 µM) containing 5 % ethanol, NC solution (2 µM) containing 0.02 % Tween-20, NC solution (2 µM) containing 5 % β-cyclodextrin and NC solution (2 µM) containing only distilled water at 20±1°C for 18 h. Untreated plums were considered as a control to compare the effects of NC formulation treatments. In the first experiment, the treated and untreated 'Tegan Blue' plums were stored at 20±1°C (ambient condition) for 10 d and at 0±1°C (90±5% RH) for 40-d. In the second experiment, the treated and untreated 'Angeleno' plums were stored at 0±1°C (90±5% RH) for 25 and 40 d. In both experiments, the changes in ethylene production (nmol kg^{-1} h^{-1}), respiration rate (mmol kg^{-1} h^{-1}), weight loss (%), fruit firmness (N), soluble solids content (SSC %), titratable acidity (TA %), SSC:TA ratio, individual sugars and organic acids (g kg^{-1}), total phenols (g kg^{-1} GAE), ascorbic acid (g kg^{-1}), anthocyanin (g kg^{-1}) and total antioxidant activity (µM kg^{-1} Trolox) were evaluated to check the postharvest quality of plums at the end of storage periods. 'Tegan Blue' and 'Angeleno' plums treated with NC exhibited significantly suppressed and delayed ethylene production regardless of formulations, reduced percent weight loss and fruit softening. In addition, the levels of SSC, SSC:TA ratio and individual sugars were

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retained lower whilst TA and organic acids concentrations were higher in NC-treated plums as compared to control. However, the effectiveness of NC on these physical and sensory quality parameters were more significant in fumigation treatment and in spray treatments with either ethanol or with Tween® 20 as compared to other NC formulations. The phytochemical compounds such as total phenols, ascorbic acid, anthocyanin and total antioxidant activity were not generally affected by NC. Overall, NC has a significant effect in delaying the ripening of 'Tegan Blue' and 'Angeleno' plums maintaining the postharvest fruit quality with intact nutritional properties. The presence of co-solvent or surfactant in preparation of spray solutions can enhance the effectiveness of ethylene antagonist NC solutions.

5.1 Introduction

Japanese plums are one of the major stone fruits with a wide range of commercial cultivars and are mainly consumed as fresh fruit (Topp et al., 2012; Crisosto and Day, 2012). The consumption of plums has been increasing with the increased awareness of their nutritional values (Gil et al., 2006). However, plums are easily perishable as they ripen rapidly after harvest restricting their storage life, and therefore their availability, as well (Khan et al., 2018). It is important to find new methods to slow down the ripening and the related processes to expand the marketable life of plums.

Fruit ripening process encompasses a series of physical and biochemical changes such as fruit softening, colour, flavour and aroma development, which ultimately leads to the spoilage of fruit. During fruit ripening, ethylene is one of the major hormones which accelerates the subsequent biochemical processes resulting in short storage life (Burg and Burg, 1965; Manganaris et al., 2008), especially in climacteric fruit like plums (Crisosto and Day, 2012). There are also suppressed-climacteric cultivars such as 'Rubyred' and 'Shiro' which are not able to produce ethylene internally like normal climacteric plum, but they respond to the external ethylene and exhibit normal ripening (Abdi et al., 1998). Both climacteric and suppressed-climacteric cultivars of plums are extremely sensitive to ethylene and responded to the very low ethylene levels of 0.01-0.1 µL $kg^{-1}h^{-1}$ (Crisosto and Kader, 2000). Controlling ethylene production and its action are two major strategies to control ripening and fruit quality during postharvest handling of plums.

Different postharvest approaches have been investigated to avoid the ethylene-induced ripening acceleration and short storage life of plums. Low-temperature storage (-1.1 to 0° C) is the most fundamental method to extend the storage life of plums for both domestic and long distant transportation (Manganaris et al., 2008; Benichou et al., 2018). In addition, the application of postharvest treatments such as polyamines, amino- ethoxyvinylglycine (AVG), nitric oxide and salicylic acid found to be effective to prolong the storage life of plum by reducing ethylene production (Khan et al., 2018). Fumigation with 1-methylcyclopropene (1-MCP) is the optimum method, presently, as it blocks the perception of ethylene rather than its biosynthesis (Valero et al., 2016; Schotsmans et al., 2009; Watkins, 2006). The combined approach of ethylene antagonist 1-MCP with the cold storage have also been developed and practised successfully to slow down ripening and to prolong the storage life of fruit (Valero et al., 2003; Benichou et al., 2018).

Nonetheless, the application of 1-MCP is restricted in open field conditions due to the gaseous nature (Sisler, 2006) and alternative ethylene antagonists which are easy to handle and as effective as 1-MCP are needed (Grichko, 2006). The new ethylene antagonist 1*H*-cyclopropa[b]naphthalene (NC) is found to be effective in blocking the ethylene action as a fumigant in some horticultural crops including plums. It interacts with the ethylene receptors in a similar mode of action like 1-MCP despite the different molecular structure. The physical properties of NC assure easy handling of this compound with the potential of different methods of application such as spray and dip treatments (Singh et al., 2018).

In this regard, the effectiveness of NC, as spray solutions or as fumigation, on ripening and postharvest quality of plums was investigated under ambient and cold storage conditions. Adjuvants are added during the preparation of spray solutions to enhance the performance of pre- or post-harvest treatments (Ho, 2013; Singh and Khan, 2012; Farag et al., 1992). In this study, ethanol, Tween® 20 and β-cyclodextrins were applied as adjuvants to enhance the performance of NC spray solutions. It was hypothesised that the ethylene antagonist NC will suppress ethylene production delaying the ripening-related quality changes. The addition of adjuvants will enhance the performance of NC spray solutions to be as effective as fumigation treatment allowing the broader use of NC.

5.2 Materials and methods

5.2.1 Experimental conditions

The impact of NC spray solutions and fumigation treatments on postharvest physiology and quality of plums (*Prunus salicina* L. cvs. 'Tegan Blue' and 'Angeleno') were evaluated by implementing two individual experiments. The plums were sourced from the Eastwind Farm (33°47'02.4"S 115°57'47.2"E), Western Australia using an air-conditioning vehicle. Tree age, rootstock and spacing of 'Tegan Blue' and 'Angeleno' plums were mentioned detail in Table 3.1, Chapter 3. The freshly harvested plums which were commercially matured, uniform in size, free from pests, diseases and physical defects were selected to expose experimental treatments. The experiments were conducted within 36 hours after harvest using a completely randomised design with three replications. The ethylene antagonist 1*H*-cyclopropa[*b*]naphthalene (NC) was prepared and supplied by the research group of Dr Alan Payne, School of Molecular and Life Sciences, Curtin University. The NC spray solutions and the fumigation treatments were prepared and applied by following the respective procedure of Choi et al., (2008) and Khan, (2007) with some modifications and the detailed procedure were mentioned in section 3.3.1.

5.2.1.1 Experiment 1: Effect of different formulations of NC on postharvest physiology and quality of 'Angeleno'

'Angeleno' plums which were commercially matured with the fruit firmness of 38.4±4 N, TSS of 15.6±0.5 % and TA of 2.8±0.2 % were collected from the Eastwind orchard on 9th March 2017. The total of 648 plums which were free from any defects were selected and divided into six lots of 108 fruit each. Then, the fruit were treated with (1) NC (2 µM) solution prepared with only distilled water, (2) NC (2 µM) solution prepared with 0.02 % Tween-20, (3) NC (2 µM) solution prepared with 5% β-cyclodextrin, (4) NC (2 µM) solution prepared with 5% ethanol, (6) NC (1 µM) fumigation and, (6) no treatments (control). For the spray solution treatments, each lot of fruit were spread into one layer on a plastic tray and sprayed thoroughly with the respective freshly prepared NC solutions using a hand sprayer (500 mL). For the fumigation treatment, the fruit were enclosed in an airtight plastic container (60 mL) along with the soda-lime to absorb carbon dioxide and a portable fan to generate an

equal amount of fumigant inside the container. All the treatments were applied at 20±1°C and kept for 18 h in airtight plastic containers as mentioned in Section 3.3.1. After removal from the plastic containers, each lot of treated and untreated plum were again split into 2 sub-lots and arranged replication wise in labelled cardboard boxes to store for 25 d and 40 d at 0±1°C and 90±5% RH. The initial weights of each replication (18 fruit per each) were taken before keeping the fruit in cold storage. At the end of each cold storage period, the final weights of each replication were checked againto calculate the physiological weight loss. Fruit quality parameters such as firmness, SSC and TA, individual sugars and organic acids, total phenols, ascorbic acid, anthocyanintotal and antioxidant capacity were also determined using 15 fruit per replication after removal from cold storage. Ethylene production and respiration rate were evaluated daily during the ripening period at 20±1°C after each storage period.

5.2.1.2 Experiment 2: Effect of different formulations of NC on postharvest physiology and quality of 'Tegan Blue'

'Tegan Blue' plum which were commercially matured with the fruit firmness of 37.1±4 N, TSS of 10.2±0.5 % and TA 1.81±0.2 % were collected from the Eastwind orchard on 14th February 2017. The selection of fruit and application of treatments were conducted following the same procedure as mentioned in experiment 1. The ethylene production and respiration rate were evaluated daily during the ripening period at 20±1°C for ambient condition and for 40 d cold storage. The initial and final weight of each replication were taken before and after the 40-d cold storage to estimate the physiological weight loss. Fruit quality parameters such as firmness, SSC and TA, individual sugars and organic acids, total phenols, ascorbic acid, total antioxidant capacity and anthocyanin contents were also determined using 15 fruit per replication at the end of 40 d cold storage.

5.2.2 Determination of physiological parameters

5.2.2.1 Ethylene production (nmol kg^{-1} h^{-1})

Ethylene production of 'Angeleno' and 'Tegan Blue' (three fruit per replication) were detected with ETD-300 ethylene detector (Sensor sense B.V, Nijmegen, The Netherlands) following the method of Azzu (2016). Ethylene production was

expressed as nmol kg^{-1} h^{-1} and the detailed procedure are mentioned in Chapter 3, Section 3.5.1.

5.2.2.2 Respiration rate (mmol CO_2 kg^{-1} h^{-1})

The respiration rate of 'Angeleno' and 'Tegan Blue' (three fruit per replication) were analysed by using a gas analyser (1450 series Gas Analyser, Servomex, United Kingdom) following the method of Khan and Singh (2008). The method for estimation of respiration rate is detailed in Chapter 3, Section 3.5.2 and the respiration rate was expressed as mmol CO_2 kg^{-1} h^{-1}.

5.2.2.3 Physiological weight loss (%) and fruit firmness (N)

To check the physiological weight loss, the initial and final weight of each replication were recorded before and after 40 d cold storage. The percent weight loss was calculated following the detailed procedure of Azzu (2016) as detailed in Chapter 3, Section 3.5.3.

The firmness was analysed by following the detailed procedure of Khan and Singh (2008) using the texture analyser (TPA Plus, AMETEK Lloyd Instruments, United Kingdom). The fruit firmness was expressed as N and the detailed procedure is described in Chapter 3, Section 3.5.5.

5.2.3 Determination of quality parameters

5.2.3.1 Soluble solids content (SSC %), titratable acidity (TA %) and SSC: TA

The SSC, TA and SSC:TA were quantified by the method previously described by Khan and Singh (2008). The detailed procedures are described in Chapter 3, Section 3.6.1. A longitudinal slice of pulp without peel was cut from individual sample fruit (15 fruit) and the juice was prepared using a blender. To estimate the percent soluble solids content of the freshly prepared juice, a portable digital refractometer (Atago-Palette PR 101, Atago Co., Itabashi-Ku, Tokyo, Japan) was used. From the same juice sample, the percent titratable acidity (malic acid equivalent) was determined by titrating against 0.1 N NaOH till the endpoint using phenolphthalein as an indicator.

5.2.3.2 Individual sugars and organic acids (g kg^{-1})

The individual sugar and organic acid were assayed by following the detailed procedure of Usenik et al. (2008) with some modifications. A reversed-phase high-performance liquid chromatography (HPLC) (Binary HPLC Pump, Waters 1525, Milford Corp., MA, USA) was used to quantify four individual sugars (glucose, fructose, sucrose and sorbitol) and four individual organic acids (malic, citric, fumaric and succinic acids). The detailed procedure of sample preparation and quantity assessment are described in Chapter 3, Section 3.6.2. The levels of individual sugars and organic acids were expressed as g kg^{-1} fresh weight.

5.2.3.3 Total phenols (g GAE kg^{-1})

The concentration of total phenols was estimated by following the method previously reported by Cantin et al. (2009) with some modifications. The procedures of total phenols extraction and assessment were detailed in Chapter 3, Section 3.6.3. The total phenolic contents were quantified at the wavelength of 750 nm by using a spectrophotometer and expressed as gallic acid equivalents gram per kilogram (g GAE kg^{-1}) fresh pulp weight.

5.2.3.4 Ascorbic acid (g kg^{-1})

The level of ascorbic acid was estimated by following the detailed procedure of Vithana (2017) at the wavelength of 760 nm using a spectrophotometer. The extraction and assessment were undertaken as previously described in Chapter 3, Section 3.6.5.

5.2.3.5 Total antioxidant capacity (mmol TEAC kg^{-1})

The total antioxidant capacity was assessed by checking the free radical scavenging capacity following the procedure previously described by Vithana (2017) and Brand-Williams et al. (1995). The spectrophotometric assay was carried out at 515 nm until the absorbance values were in the range of 0.6-0.7. The preparation of the free radical solution and the extraction and assessment of antioxidant compounds were mentioned in Chapter 3, section 3.6.5 in detail. The total antioxidant capacity was calculated by standardizing with the standard curve of Trolox and mentioned as mM Trolox equivalent antioxidant activity per kg (mmol TEAC kg^{-1}).

5.2.3.6 Total Anthocyanin (g kg^{-1})

The total anthocyanin content in the peel was estimated by following the procedure of Whales and Singh (2007) based on the method previously described by Siegelman and Hendricks (1958). The anthocyanin extraction and assessment were carried out as detailed in Chapter 3, Section 3.6.5 by using a spectrophotometer at the wavelength of 530 nm. The total anthocyanin content was standardised with the idaein chloride (Cyanidin-3-gluciside) standard and expressed as g kg^{-1} of fresh peel weight.

5.2.4 Statistical analysis

The data were analysed by using Genstat software (13th edition), Genstat realease 13.1, VSN International Ltd., UK. The treatment effects were compared by calculating the least significant differences (LSD) at ($P \leq 0.05$). The data for ethylene production and respiration rate were subjected to two-way analysis of variance while all the rest of the parameters were subjected to one-way ANOVA. The multiple mean comparison was calculated by using Duncan's Multiple Range Test.

5.3 Results

5.3.1 Ethylene production (nmol kg^{-1} h^{-1})

In general, ethylene production of 'Tegan Blue' plum was 23-32 fold higher and the climacteric peak onset was 5-d earlier as compared to that of 'Angeleno' (Fig. 5.1). NC significantly suppressed and delayed the climacteric ethylene production of 'Tegan Blue' and 'Angeleno' plum regardless of the formulations (Fig. 5.1).

The climacteric ethylene peak of NC fumigated 'Tegan Blue' plum were exhibited three days later (on 9th day of ripening) as compared to control, whilst the peaks of the fruit treated with the rest of NC formulations were recorded one day later (on 7th day of ripening) after 0 d cold storage (Fig. 5.1 A). After 40 d cold storage, the peak of 'Tegan Blue' plum treated with NC fumigation and NC solution containing ethanol were exhibited two days later (on 6th day of ripening), the peak of the fruit treated with NC solution containing β-cyclodextrin was one day earlier (on 3th day of ripening) and the peak of the fruit treated with NC solutions containing Tween® 20 was on the same

day (on 4th day of ripening) as compared to control (Fig. 5.1 B). The climacteric ethylene concentration of 'Tegan Blue' plum treated with NC fumigation (3.9 and 1.5 fold), NC solutions containing ethanol (2.8 and 1.3 fold), Tween® 20 (1.7 and 1.5 fold), β-cyclodextrin (1.1 and 1.2 fold) and only distilled water (1.3 fold each) were significantly suppressed as compared to control after 0 and 40 d cold storage, respectively (Fig. 5.1 A, B).

The climacteric ethylene peaks of untreated 'Angeleno' plum were recorded on 13th and 9th day of ripening after 25 and 40 d cold storage respectively, while the peaks of the fruit treated with NC formulations were not clearly exhibited till the end of ripening periods, except the fruit treated with NC solutions containing distilled water, and β-cyclodextrin (Fig. 5.1 C, D). When on averaged, ethylene concentration of 'Angeleno' plum treated with NC fumigation (5.1 and 2.7 fold), NC solutions containing ethanol (5.1 and 3.3 fold), Tween® 20 (3.7 and 2.9 fold), β-cyclodextrin (3.7 and 1.8 fold) and only distilled water (1.2 and 1.6 fold each) were significantly lowered as compared to control after 25 and 40 d cold storages, respectively (Fig. 5.1 C, D).

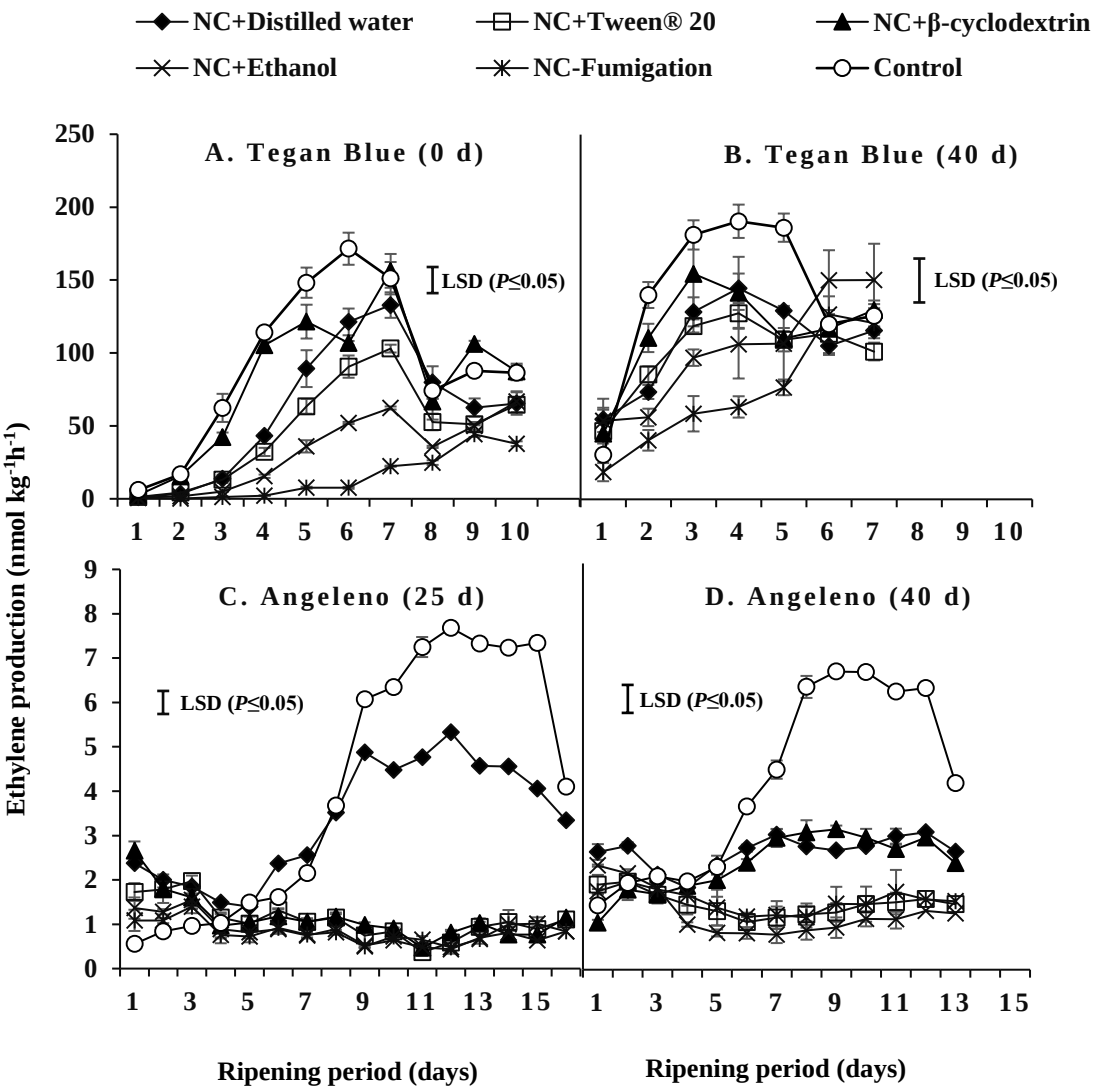

Fig. 5. 1 Effect of NC formulated as spray solutions (NC + Distilled water, NC + Tween® 20, NC + β-cyclodextrin and NC + Ethanol) and as fumigant (NC-fumigation) on ethylene production of 'Tegan Blue' plum (stored for 0 and 40 d) and 'Angeleno' (stored for 25 and 40 d) at 1°C. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for ethylene production of Tegan Blue (0 d): treatments (tr) =5.7, d after storage (d) =7.3 and their interaction (tr x d) =17.9, Tegan Blue (40 d): tr=15.2, d =16.4, tr x d =40.1, Angeleno (25 d): tr =0.13, d =0.21, tr x d =0.51, Angeleno (40 d): tr =0.18 , d =0.26 , tr x d = 0.63.

5.3.2 Respiration rate (mmol kg^{-1} h^{-1})

In general, the respiration rate of 'Tegan Blue' plum (0.9-3.1 mmol kg^{-1} h^{-1}) exhibited increasing trend while that of 'Angeleno' plum (0.8-1.7 mmol kg^{-1} h^{-1}) were found to be steady throughout the ripening period (Fig. 5.2). The climacteric respiratory peak of 'Tegan Blue' plum treated with NC fumigation (1.8 mmol kg^{-1} h^{-1}) and NC solutions containing distilled water (2.1 mmol kg^{-1} h^{-1}) were exhibited one day later than control on 9^{th} day whilst that of NC solutions containing ethanol (1.78 mmol kg^{-1} h^{-1}) and β-cyclodextrin (1.96 mmol kg^{-1} h^{-1}) were revealed on 10^{th} day of ripening period after 0 d cold storage. The peak of untreated 'Tegan Blue' plum (2.3 mmol kg^{-1} h^{-1}) was observed on the same day as of 'Tegan Blue' plum treated with NC solution containing Tween® 20 (2.2 mmol kg^{-1} h^{-1}) on 8^{th} day of ripening period after 0 d cold storage (Fig 5.2 A). After 40 d cold storage, the respiratory climacteric peaks of treated and untreated 'Tegan Blue' plum were noted on 7^{th} day irrespective of the formulations (Fig. 5.2 B). The lowest respiration rate (2 mmol kg^{-1} h^{-1} each) were recorded in the fruit treated with NC fumigation and NC solution containing ethanol although they were not significantly different as compared to control and other NC formulations.

The respiratory peak of untreated 'Angeleno' plums (1.6 mmol kg^{-1} h^{-1}) were three days earlier than of the fruit treated with NC fumigation (1.55 mmol kg^{-1} h^{-1}) after 25 d cold storage whilst the peaks of other formulations were not clearly observed (Fig 5.2 C). The respiratory peak of control fruit (1.7 mmol kg^{-1} h^{-1}) was recorded on 6^{th} day of ripening period along with other NC treated fruit. The minimum average level of respiration was recorded in fruit treated with NC solution containing Tween® 20, followed by NC solution containing ethanol, which were 1.4 and 1.3 fold, respectively lower than control after 40 d cold storage (Fig. 5.2 D). The respiration rates of the fruit treated with the rest of NC formulations were significantly the same as compared to control after 40 d cold storage.

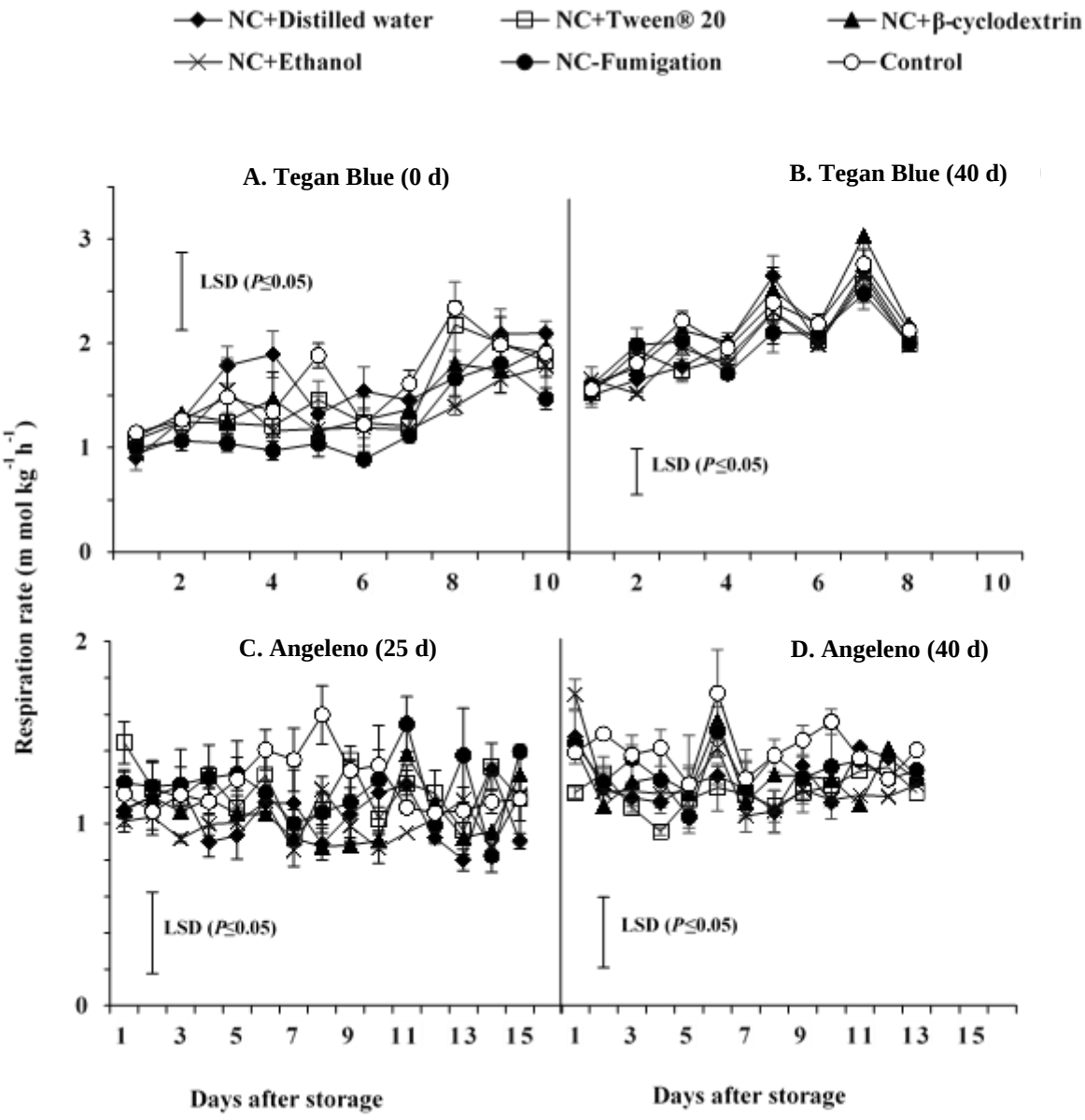

Fig. 5. 2 Effect of NC formulated as spray solutions (NC+Distilled water, NC+Tween® 20, NC+β-cyclodextrin and NC+Ethanol) and as fumigant (NC-fumigation) on respiration rate of 'Angeleno' (stored for 25 and 40 d) and 'Tegan Blue' plum (stored for 0 and 40 d) at 1°C. LSD bars represent treatments (tr) x days after storage (d). Vertical bars describe SE of means of three replicates. LSD values for respiration rate of Tegan Blue (0 d): treatments (tr) =0.24, d after storage (d) =0.30 and their interaction (tr x d) =ns, Tegan Blue (40 d): tr = ns, d =0.18 , tr x d =ns, Angeleno (25 d): tr =0.12, d =ns, tr x d =ns, Angeleno (40 d): tr =0.11, d =0.16, tr x d = ns.

5.3.3 Physiological weight loss (%)

The fruits, ‘Angeleno’ plum stored for 25 days and ‘Tegan Blue’ plum stored for 40 days, treated with NC formulations maintained significantly higher physiological weight as compared to the untreated fruit regardless of adjuvants (Table 5.1). ‘Angeleno’ plum treated with NC fumigation, NC solutions containing ethanol, and Tween® 20 maintained significantly the lowest weight loss (2.3 fold each), followed by NC solutions containing β-cyclodextrin, and distilled water (1.2 fold each) when compared to control after 25 d cold storage. Weight loss of the fruit exposed to NC fumigation, NC solutions containing ethanol, and Tween® 20 were significantly reduced (2.0, 1.6 and 1.3 fold respectively) as compared to the weight loss of control, which was significantly the same as of other NC formulations after 40 d cold storage. The weight loss of ‘Tegan Blue’ plum treated with NC fumigation, NC solutions containing ethanol, distilled water, Tween® 20 and β-cyclodextrin were significantly lower (2.0, 1.6, 1.5, 1.4 and 1.3 fold, respectively) as compared to control, although they were not significantly different among each other after 40 d cold storage.

5.3.4 Firmness (N)

Fruit firmness of ‘Angeleno’ plum treated with NC formulations were significantly retained higher as compared to control irrespective of the adjuvants after 25 days storage. The firmness of ‘Angeleno’ plum stored for 40 d at 1°C were comparatively higher than of ‘Tegan Blue’ (Table 5.1). The untreated ‘Angeleno’ plums were observed to be significantly softest (23.6 N) as compared to other fruit treated with NC formulations after 25 d cold storage. The significantly highest fruit firmness was recorded in the fruit treated with NC fumigation (30.3 N) followed by NC solutions containing ethanol (28.9 N) and Tween® 20 (26.5 N) after 25 d cold storage. The firmness of the fruit treated with NC solutions containing β-cyclodextrin and distilled water were significantly higher (1.0 and 1.1 fold respectively) as compared to control but significantly lower than the other NC formulations. After 40 d cold storage, ‘Angeleno’ plum exposed to NC fumigation and NC solution containing ethanol maintained the significantly highest firmness (28.0 and 26.1 N, respectively). The firmness of the fruit sprayed with NC solution containing Tween® 20 was significantly higher as compared to control (1.2 fold), NC solutions containing β-cyclodextrin (1.3 fold), and distilled water (1.2 fold) after 40-day cold storage.

‘Tegan Blue’ plum treated with NC fumigation, NC solutions containing Tween® 20, and ethanol exhibited significantly higher firmness (1.6, 1.5 and 1.4 fold respectively) as compared to control which had significantly the same firmness as of the fruit sprayed with NC solutions containing distilled water and β-cyclodextrin after 40 d cold storage.

Table 5. 1 Effect of different formulations of ethylene antagonist NC on the percent weight loss and the firmness of ‘Angeleno’ and ‘Tegan Blue’ plum stored for 25 d and 40 d at cold storage (1 °C)

	Treatment	Weight Loss (%)		Firmness (N)	
		25 d	40 d	25 d	40 d
Angeleno	Control	5.7±0.1c	4.7±0.1c	23.6±0.2a	20.2±0.3a
	NC+Distilled water	4.7±0.1b	4.4±0.1c	25.2±0.1b	19.7±1.3a
	NC+Tween® 20	2.6±0.3a	3.5±0.1b	26.5±0.2c	24.2±0.4b
	NC+β-cyclodextrin	4.6±0.1b	4.2±0.2c	24.7±0.1b	18.2±0.7a
	NC+Ethanol	2.5±0.1a	2.9±0.2a	28.9±0.3d	26.1±0.1c
	NC-Fumigation	2.5±0.1a	2.3±0.1a	30.3±0.2e	28.0±0.1c
	LSD ($P \leq 0.05$)	0.49**	0.52**	0.81**	1.97**
Tegan Blue	Control		5.5±0.5c		12.3±0.8a
	NC+Distilled water		3.6±0.2ab		12.3±0.4a
	NC+Tween® 20		4.0±0.2ab		19.1±1.1bc
	NC+β-cyclodextrin		4.1±0.3b		13.0±0.6a
	NC+Ethanol		3.5±0.4ab		17.0±1.1b
	NC-Fumigation		2.8±0.1a		19.4±1.4c
	LSD ($P \leq 0.05$)		1.19*		2.29**

n = 3 replicates (15 fruit for weight loss and 12 fruit for firmness per replication), ± 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 within the same columns are not significantly different. The data for each plum cultivars and storage period were analysed individually.

5.3.5 SSC (%), TA (%) and SSC:TA

The SSC, TA and SSC:TA of ‘Angeleno’ and ‘Tegan Blue’ plums were generally affected by NC formulations, except NC solution with distilled water after 25 and 40 d cold storages (Table 5.2). The SSC percent of ‘Angeleno’ plum treated with NC fumigation, NC solutions containing ethanol, β-cyclodextrin, and Tween® 20 were significantly lower (1.3, 1.3, 1.2 and 1.2 fold respectively) as compared to control after 25-day cold storage. After 40 d cold storage, the fruit treated with NC fumigation, NC solutions containing ethanol, and Tween® 20, showed significantly reduced level of SSC (1.2 fold each) as compared to control and rest of NC formulations which were not significantly different from each other. The similar trend of reduction in SSC:TA ratio of ‘Angeleno’ as affected by NC formulations was observed after 25 and 40 d cold storages. The TA percent of ‘Angeleno’ plum treated with NC fumigation, NC solutions containing ethanol, and Tween® 20 were significantly maintained ≈1.2 and 1.3 fold higher than control and other NC formulations after 25 and 40d cold storage, respectively.

The SSC content and SSC:TA ratio of ‘Tegan Blue’ plum exposed to NC formulations, except NC solution with distilled water, were significantly reduced up to 1.3 and 1.7 fold as compared to control after 40 d cold storage. The fruit treated with NC fumigation, and NC solution containing Tween® 20 maintained significantly higher TA percent (1.2 and 1.3 fold, respectively) than control which was not significantly different from the rest of NC formulations after 40 d cold storage (Table 5.2).

Table 5. 2 Effect of different formulations of ethylene antagonist NC on the SSC, TA and SSC/TA ratio of ‘Angeleno’ and ‘Tegan Blue’ plum stored for 25 d and 40 d at cold storage (1 °C)

	Treatment	SSC (%)		TA (%)		SSC/TA (%)	
		25 d	40 d	25 d	40 d	25 d	40 d
Angeleno	Control	15.2±0.1c	15.4±0.1c	0.87±0.0a	0.78±0.02ab	17.49±0.7b	19.74±0.6bc
	NC+Distilled water	14.9±0.1c	15.1±0.1c	0.80±0.0a	0.72±0.02a	18.57±0.1b	21.16±0.4c
	NC+Tween® 20	13.1±0.03b	13.4±0.1ab	1.05±0.02b	0.94±0.0c	12.46±0.2a	14.29±0.1a
	NC+β-cyclodextrin	13.2±0.3b	14.4±0.4bc	0.76±0.04a	0.80±0.03b	17.45±0.5b	18.01±1.2b
	NC+Ethanol	11.9±0.1a	13.1±0.4a	1.01±0.0b	0.96±0.02c	11.87±0.1a	13.67±0.4a
	NC-Fumigation	12.2±0.1a	12.7±0.6a	1.07±0.05b	0.98±0.02c	11.42±0.5a	12.95±0.7a
	LSD ($P \leq 0.05$)	0.52**	1.05**	0.12**	0.08**	1.71**	2.31**
Tegan Blue	Control		12.40±0.85b		1.27±0.03a		9.79±0.9c
	NC+Distilled water		11.33±0.62ab		1.30±0.04a		8.81±0.7bc
	NC+Tween® 20		9.83±0.47a		1.68±0.06c		5.87±0.1a
	NC+β-cyclodextrin		9.43±0.14a		1.36±0.02ab		6.93±0.1ab
	NC+Ethanol		9.73±0.22a		1.36±0.04ab		7.17±0.3ab
	NC-Fumigation		9.77±0.15a		1.47±0.00b		6.63±0.1a
	LSD ($P \leq 0.05$)		1.93*		0.15*		2.01*

n = 3 replicates (15 fruit per replication), ± 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 the similar letter are not significantly different within the columns. The data for each plum cultivars and storage period were analysed individually. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

5.3.6 Individual sugars (g kg^{-1})

The glucose, fructose, sucrose and sorbitol were identified, and the fructose level was tendentially higher than other individual sugars in both 'Angeleno' and 'Tegan Blue' plums (Table 5.3). In general, the fruit treated with NC maintained tendentially lower levels of glucose, fructose and sorbitol while the level of sucrose was higher as compared to untreated one after 25 and 40 d cold storages. The glucose levels of 'Angeleno' plum treated with NC formulations, except NC solution with distilled water, were significantly reduced up to 21 % as compared to control after 25 and 40 d cold storages. The fructose content of NC-fumigated fruit was significantly the lowest (189.1 g kg^{-1}), followed by that of the fruit sprayed with NC solutions which were not significantly different from each other but significantly lower ≈1.2 fold as compared to control after 25 d cold storage. The level of fructose in the fruit treated with NC formulations, except NC solution with distilled water, were significantly lower up to 1.1 fold as compared to control after 40 d cold storage. The sucrose content of the fruit treated with NC solution containing ethanol was significantly higher (1.1 fold) as compared to control and rest of NC formulations after 40 d cold storage. The sorbitol levels of the fruit treated with NC solution containing ethanol, Tween® 20 and NC fumigation were significantly higher (1.7, 1.4 and 1.3 fold, respectively) as compared to control which was not significantly different from other NC formulations after 25 d cold storage. The sucrose content of the fruit treated with NC fumigation, NC solutions containing β-cyclodextrin, and Tween® 20, were significantly reduced (1.6, 1.5 and 1.4 fold, respectively) as compared to control which retained significantly the same amount of sorbitol as in the fruit treated with NC solutions containing ethanol (73.9 g kg^{-1}) and distilled water (67.0 g kg^{-1}).

The levels of glucose and fructose of 'Tegan Blue' plum treated with NC solution containing Tween® 20 were maintained significantly lower (1.3 and 1.5 fold, respectively) as compared to control, whilst that of the fruit treated with other NC formulations were not significantly different from control (52.23 and 61.96 g kg^{-1}, respectively) after 40 d cold storage. The sucrose contents of 'Tegan Blue' plum were not significantly affected by NC formulations. The sorbitol content of NC fumigated fruit was significantly lower (1.5 fold) as compared to control whilst that of the fruit

sprayed with NC spray solutions remained the same as in control (27.8 g kg^{-1}) after 40 d cold storage.

5.3.7 Individual organic acids (g kg^{-1})

The malic, citric and succinic acids were identified in both ‘Angeleno’ and ‘Tegan Blue’ plum, while fumaric acid was detected only in ‘Angeleno’ after 25 and 40 d storage (Table 5.4). In both plums, malic acid concentrations were comparatively higher than other individual organic acids. NC formulations maintained tendentially higher malic, citric and succinic acid contents in ‘Angeleno’ and ‘Tegan Blue’ plum as compared to control after 25 and 40 d cold storage (Table 5.4).

‘Angeleno’ plum treated with NC fumigation, NC solutions containing ethanol, and Tween® 20, retained significantly higher malic acid contents up to 30 % as compared to untreated ones which were not significantly different with rest of NC formulations after 25 and 40 d cold storages. The citric and fumaric acid level of ‘Angeleno’ plum were not significantly affected by NC formulations after 25 and 40 d cold storages, except citric acid content of the fruit treated with NC solution containing ethanol which was significantly higher (1.2 fold) than control. The succinic acid contents of the fruit treated with NC formulations, except NC solution with distilled water, were significantly higher up to 1.3 fold as compared to control after 25 d cold storage. After 40 d cold storage, the fruit exposed to NC fumigation and NC solutions containing ethanol exhibited significantly higher (1.2 fold each) succinic acid as compared to control and rest of NC formulations which were not significantly different from each other. The malic and succinic acid of ‘Tegan Blue’ plum treated with NC formulations were retained significantly higher as compared to control irrespective of the adjuvants, whilst the citric acid was not affected by NC formulations after 40 d cold storage. All the NC formulations showed significantly the same effect on the malic acid, as well as succinic acid content of ‘Tegan Blue’ plum after 40 d cold storage.

Table 5. 3 Effect of different formulations of ethylene antagonist NC on the individual sugars of 'Angeleno' and 'Tegan Blue' plum stored for 25 d and 40 d at cold storage (1°C)

	Treatment	Glucose (g kg^{-1})		Fructose (g kg^{-1})		Sucrose (g kg^{-1})		Sorbitol (g kg^{-1})	
		25 d	40 d	25 d	40 d	25 d	40 d	25 d	40 d
Angeleno	Control	174.1±1.6c	195.9±2.3c	252.4±1.8e	278.9±3.2c	23.59±1.1a	33.73±0.4a	71.24±3.7c	77.20±1.9d
	NC+Distilled water	161.9±3.9bc	190.1±2.2bc	232.4±5.8bd	273.5±3.2bc	26.20±2.1a	33.41±0.3a	63.38±2.3bc	67.00±3.7bcd
	NC+Tween® 20	149.2±5.4ab	180.5±1.6a	211.5±6.9bc	257.1±1.9a	25.01±1.2a	32.12±0.1a	59.98±4.6bc	56.20±1.9abc
	NC+β-cyclodextrin	155.5±2.8ab	183.0±1.9ab	219.1±5.7bcd	260.3±3.5ab	28.03±2.5ab	33.63±0.3a	52.44±2.9ab	50.16±4.3ab
	NC+Ethanol	147.0±2.7a	186.7±1.8ab	211.5±4.7b	259.5±3.3ab	24.00±1.2a	36.06±0.8b	42.76±2.1a	73.88±8.0cd
	NC-Fumigation	143.5±1.1a	180.7±3.1a	189.1±1.2a	256.6±4.7a	34.95±2.8b	33.65±0.7a	52.91±2.5ab	47.00±3.8a
	LSD ($P \leq 0.05$)	13.42*	7.61*	19.88**	13.69*	ns	1.81*	12.95*	17.7*
Tegan Blue	Control		52.23±1.1b		61.96±1.1b		25.95±1.9		27.80±1.9bc
	NC+Distilled water		48.89±1.1ab		53.54±0.6b		28.35±2.3		28.77±2.0bc
	NC+Tween® 20		40.67±0.6a		40.63±3.2a		28.52±2.9		21.25±1.1ab
	NC+β-cyclodextrin		51.89±2.7b		53.99±1.5b		36.73±4.4		29.67±2.0bc
	NC+Ethanol		52.64±1.4b		62.17±4.2b		27.32±2.6		32.50±0.6c
	NC-Fumigation		47.09±3.2ab		57.23±3.6b		23.75±3.3		18.17±3.5a
	LSD ($P \leq 0.05$)		7.89*		11.19*		ns		7.89*

n = 3 replicates (15 fruit per replication), ± 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. The data for each plum cultivars and storage period were analysed individually. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 5. 4 Effect of different formulations of ethylene antagonist NC on the individual organic acids of ‘Angeleno’ and ‘Tegan Blue’ plum stored for 25 d and 40 d at cold storage (1°C)

	Treatment	Malic acid (g kg^{-1})		Citric acid (g kg^{-1})		Fumaric acid (g kg^{-1})		Succinic acid (g kg^{-1})	
		25 d	40 d	25 d	40 d	25 d	40 d	25 d	40 d
Angeleno	Control	26.29±0.2a	20.56±0.5a	0.64±0.0ab	0.93±0.0ab	0.22±0.0	0.30±0.0	4.02±0.2a	4.43±0.0ab
	NC+Distilled water	26.21±0.3a	21.45±0.8ab	0.61±0.1a	0.93±0.0ab	0.21±0.0	0.30±0.01	4.49±0.1ab	4.09±0.2a
	NC+Tween® 20	29.85±1.1c	24.20±1.5bc	0.62±0.1a	0.96±0.1ab	0.22±0.0	0.29±0.0	4.68±0.1b	4.65±0.1b
	NC+β-cyclodextrin	26.78±0.1ab	21.06±0.7a	0.76±0.0ab	0.88±0.0a	0.22±0.0	0.29±0.0	4.81±0.1bc	4.21±0.1ab
	NC+Ethanol	28.56±0.4bc	26.72±0.2c	0.84±0.0b	1.15±0.1c	0.21±0.0	0.29±0.0	5.22±0.1c	5.45±0.1c
	NC-Fumigation	28.64±0.7bc	25.93±0.3c	0.76±0.1ab	1.06±0.0bc	0.22±0.0	0.31±0.01	4.87±0.1bc	5.12±0.1c
	LSD ($P \leq 0.05$)	2.11*	2.85*	ns	0.15*	ns	ns	0.49*	0.44**
Tegan Blue	Control		18.5±0.17a		0.35±0.02a				3.4±0.03a
	NC+Distilled water		21.5±0.65b		0.35±0.01a				3.8±0.07b
	NC+Tween® 20		23.8±0.22c		0.40±0.01b				3.8±0.07b
	NC+β-cyclodextrin		22.3±0.63bc		0.36±0.0ab				3.8±0.14b
	NC+Ethanol		23.7±0.49c		0.37±0.0ab				3.9±0.07b
	NC-Fumigation		23.3±0.22c		0.38±0.0ab				3.9±0.04b
	LSD ($P \leq 0.05$)		1.68**		ns				0.29*

n = 3 replicates (15 fruit per replication), ± 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 the similar letter are not significantly different within the columns. The data for each plum cultivars and storage period were analysed individually.

5.3.8 Total phenols (g GAE kg^{-1})

The phenolic contents of ‘Angeleno’ plum treated with NC formulations were not significantly different from the untreated plums in both storage periods. The fruit treated with NC solution containing Tween® 20 retained the lowest phenolic content (61.0 and 62.8 g GAE kg^{-1}, respectively) after 25 and 40 d cold storages. ‘Tegan Blue’ plum treated with NC formulations, except NC solution with distilled water, retained lower level of phenolic content up to 1.4 fold as compared to control after 40 d cold storage and the lowest level of phenolic concentration was recorded in ‘Tegan Blue’ plum treated with NC solution containing Tween® 20.

5.3.9 Ascorbic acid (g kg^{-1}), antioxidant capacity (mmol TEAC kg^{-1}) and total anthocyanin (g kg^{-1})

The ascorbic acid content and the antioxidant capacity of ‘Angeleno’ and ‘Tegan Blue’ plum treated with NC formulations were maintained significantly the same as of untreated fruit after 25 and 40 d cold storages. The anthocyanin concentrations of ‘Angeleno’ plum treated with NC formulations remained similar to the untreated plum after 25 d cold storage, however, the levels in fruit treated with NC fumigation, NC solutions containing ethanol and Tween® 20 were lower ≈1.2 fold than control. The fruit treated with NC formulations, except NC solution containing distilled water, retained a lower level of anthocyanin up to 1.4 fold as compared to control, but significantly the same among each other after 40 d cold storage. ‘Tegan Blue’ plum treated with NC formulations exhibited significantly the same levels of anthocyanin as compared to control after 40 d cold storage, however, the NC fumigated ‘Tegan Blue’ plum retained a lower level of anthocyanin (27.32 g kg^{-1}) compared to the rest of NC-treated fruit.

Table 5. 5 Effect of different formulations of ethylene antagonist NC on the total phenols, ascorbic acid and antioxidant contents of 'Angeleno' and 'Tegan Blue' plum stored for 25 d and 40 d at cold storage (1°C)

	Treatment	Total phenols (g GAE kg^{-1})		Ascorbic acid (g kg^{-1})		Antioxidant activity (mmol TEAC kg^{-1})		Anthocyanins (g kg^{-1})	
		25 d	40 d	25 d	40 d	25 d	40 d	25 d	40 d
Angeleno	Control	86.58±6.5	96.06±2.9	10.96±0.4	12.17±1.2	14.15±1.0	14.58±0.7	33.71±1.4	33.12±1.4b
	NC+Distilled water	79.47±5.6	99.18±7.7	10.49±0.1	11.98±0.5	13.62±1.2	16.45±1.2	31.07±1.7	33.46±1.3b
	NC+Tween® 20	61.00±0.9	62.75±3.5	10.12±0.2	12.33±0.5	13.99±0.5	14.22±1.2	28.70±1.6	25.20±0.5a
	NC+β-cyclodextrin	75.47±3.6	75.47±0.6	10.84±0.5	10.26±0.2	14.22±0.7	15.14±0.5	33.17±1.4	26.52±1.3a
	NC+Ethanol	75.47±5.2	76.97±4.4	10.51±0.9	12.12±0.2	15.39±0.3	17.35±2.8	30.30±1.1	23.79±0.4a
	NC-Fumigation	72.73±2.5	72.98±3.4	10.75±0.8	11.19±0.4	15.73±2.2	18.74±2.6	28.61±2.0	27.25±2.3a
	LSD ($P \leq 0.05$)	ns	ns	ns	ns	ns	ns	ns	5.71*
Tegan Blue	Control		80.84±5.9		14.24±0.2		20.88±1.5		30.16±0.9
	NC+Distilled water		90.10±3.5		12.61±0.2		17.25±1.2		34.13±0.7
	NC+Tween® 20		75.85±3.6		15.55±0.7		18.93±1.5		34.83±1.5
	NC+β-cyclodextrin		93.69±3.7		16.46±0.4		16.92±0.9		31.59±2.6
	NC+Ethanol		71.11±3.1		16.62±2.2		16.01±1.1		32.34±1.9
	NC-Fumigation		73.23±1.6		15.08±0.8		20.91±1.3		27.32±1.6
	LSD ($P \leq 0.05$)		ns		ns		ns		ns

n = 3 replicates (15 fruit per replication), ± 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. The data for each plum cultivars and storage period were analysed individually. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

5.4 Discussion

5.4.1 Ethylene production

In the present study, 'Tegan Blue' and 'Angeleno' plum exhibited two distinct behaviours of climacteric and suppressed-climacteric ethylene production as previously reported by Abdi et al. (1997) in climacteric 'Shiro' and suppressed-climacteric 'Rubyred' plums where ethylene production of the former was 45 fold higher than the latter one. As expected, NC suppressed and delayed the climacteric ethylene production of 'Tegan Blue' and 'Angeleno' plum irrespective of the formulations. The suppression of ethylene production may be caused by NC binding to the metal cofactor at the ethylene receptor site, subsequently blocking the binding of ethylene to its receptors as proposed by Musa (2016) and Khan (2014). In both tested plum cultivars, the ethylene suppression by NC was more obvious in NC fumigation followed by NC solutions with ethanol and with Tween® 20 as compared to other NC spray solutions. When NC was applied as a fumigant in an airtight container for 18 h, more amounts of NC might have diffused to the fruit tissue due to the longer exposure time and showed better performance than the spray solutions which have only ≈ 5-10 min of exposure time. Similarly, Sisler et al. (2009) reported that, when compared to gaseous cyclopropenes, the higher amount of cyclopropene salt solutions and longer exposure time were required for inhibition of ethylene action in fruit ripening of banana. The comparative higher ethylene suppression by NC solution with ethanol compared to other solutions and control can be rationalised by ethanol increasing the solubility and delivery of NC to the fruit tissue thereby enhancing the performance of NC (Fig. 5. 3 A). This is consistent with the findings of Farag et al. (1992) who also found that the presence of ethanol in ethephon spray solution enhances the efficacy of ethephon to increase anthocyanin accumulation in cranberry. Similarly for NC in Tween® 20 solution, the hydrophilic head and the lipophilic tail of Tween® 20 might have helped to increase the solubility of NC in aqueous solution and the permeability of NC through the fruit surface cuticle, allowing the higher infiltration of aqueous NC (Fig. 5. 3 B). This might be the reason for the enhancement of NC performance by the presence of Tween® 20. It has been reported that the pre-treatment with (0.2%) surfactant before the postharvest calcium dipping increased the calcium uptake of peel and pulp in 'Haden' mango (Singh et al., 2000) and in 'Golden Delicious' apple (Roy et al., 1996). For NC solution with β-

cyclodextrin, the possibility of relatively higher antagonist effect on ethylene production is that the β-cyclodextrin and NC complex (host-guest) formed successfully and thus increased the solubility of NC in spray solution allowing the slow release of NC. The beneficial modifications of guest molecules by cyclodextrins have been documented by Del Valle, (2004). The lower efficacy in NC solution with distilled water as compared to other solutions may be due to the lack of adjuvants which can promote the performance of NC as mentioned above. The variation in ethylene antagonist effect of NC depending on the formulations also influenced other ethylene related postharvest fruit quality parameters in the present study.

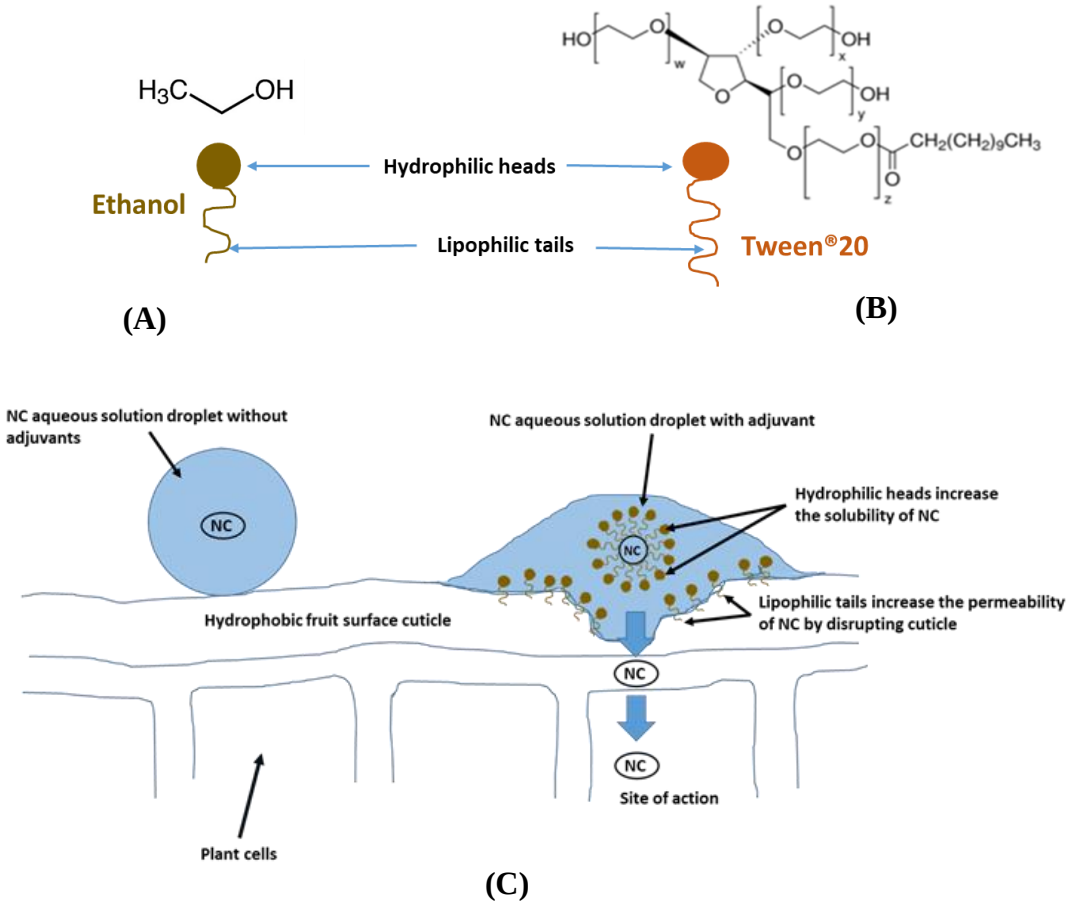

Fig. 5. 3. Structural features of ethanol (A) and Tween® 20 (B) and the proposed method of the enhancement of the performance of NC (C).

5.4.2 Respiration rate

The average respiration rate of 'Angeleno' plum was almost half of 'Tegan Blue' plum and their respiration rates were steady during the ripening periods (at 20°C) after the cold storages indicating the suppressed-climacteric respiratory nature. This is in agreement with Abdi et al., (1997) who reported that the suppressed-climacteric plum 'Rubyred' produces only half the average CO_2 of climacteric plum 'Shiro' and postharvest CO_2 production of the former variety at 20°C was observed to be constant. The delayed onset of respiratory peaks and reduction in CO_2 production of NC-treated 'Tegan Blue' plums confirmed the antagonist effect of NC. The similar reduction in postharvest respiration rate by ethylene antagonist were documented in 'Joanna Red' (Minas et al., 2015), 'President' (Valero et al., 2003) and in 'Royal Zee' plum (Dong et al., 2002). However, the respiration rate of 'Angeleno' in the present study were not affected by the ethylene antagonist NC in the same way as of 'Angeleno' plum treated with 0.5 µL L^{-1} for 24 h at 20°C (Minas et al., 2015) and of 'Black Amber' plum treated with different concentrations of 1-MCP and stored for 45 d at 0°C (Ozkaya et al., 2010). The different behaviour of ripening in 'Tegan Blue' and 'Angeleno' plums ascribed to the variation in responses of respiration rates to NC.

5.4.3 Physiological weight loss and firmness

NC found to be effective in reducing the physiological weight loss and fruit softening of both 'Tegan Blue' and 'Angeleno' plum irrespective of the formulations. However, the retention of fruit weight and firmness were varied depending on the NC formulations. The fruit weight loss is related to the cumulative loss of water which mainly depends on the vapour pressure deficit during the storage (Valero and Serrano, 2010a). The reduction in firmness is due to the increasing activities of softening enzymes and Khan and Singh (2007a) confirmed that the ethylene antagonist 1-MCP can suppress the activity of these enzymes in 'Tegan Blue' plum. It has also been reported that the weight loss and fruit softening were reduced by the ethylene antagonist at different concentrations in 'Harrow Sun' plum (Manganaris et al., 2007), in 'Santa Rosa' and 'Golden Japan' plums (Martinez-Romero et al., 2003), in 'President' plum (Valero et al., 2003). In this study, the firmness retention in 'Angeleno' plum was comparatively higher than 'Tegan Blue' after 40 d cold storage

exhibiting the suppressed-climacteric behaviour as previously observed by Minas et al. (2015), Singh and Khan (2010) and Candan et al. (2008).

5.4.4 SSC, TA and SSC:TA

The level of SSC, TA and SSC:TA are important quality parameters for the consumer acceptance in plums (Crisosto and Day, 2012) and they are reported to be ethylene dependent quality parameters in apple (Defilippi et al., 2004). In the present study, NC formulations, except NC solution with distilled water, maintained lower SSC percent and SSC:TA ratio and, relatively higher TA percent in both 'Angeleno' and 'Tegan Blue' plums. This might be the consequential effect of retardation in ethylene production by NC. The similar influence of ethylene antagonist on these quality attributes was previously reported by Velardo-Micharet et al. (2017) in 'Songold' plum which was treated with 1-MCP 0.6 µL L^{-1} for 24 h and stored at 0 and 8 °C. In contrast, the SSC percent and SSC:TA ratio in 'Black Amber', 'Red Lane' and 'Royal Zee' plums were not influenced by the postharvest application of 1-MCP although the TA percent were significantly reduced (Minas et al., 2013; Dong et al., 2002). These variations in response to ethylene antagonists may be due to the differences in cultivar, harvest maturity, storage temperature and the concentration applied (Watkins, 2006).

5.4.5 Individual sugars and organic acids

Four individual sugars glucose, fructose, sucrose and sorbitol, and four individual organic acids malic, citric, succinic and fumaric acids were identified in 'Angeleno' and 'Tegan Blue' plum. Amongst the identified sugars and organic acids, fructose and malic acid were the predominant ones in both cultivars. This is consistent with the findings of Bae et al. (2014) in 'Formosa' plum, Wang et al. (2014) in 'Angeleno' plum, Singh et al. (2009) in 'Amber Jewel' and 'Black Amber' plums. The relative accumulation of individual sugars and organic acids in 'Angeleno' and 'Tegan Blue' plums were varied depending on the cultivars as previously reported by Singh and Singh (2008) in 'Blackamber', 'Amber Jewel' and 'Angeleno'. The level of glucose, fructose and sorbitol of NC treated fruit were significantly lower whilst the level of malic and succinic acids was higher as compared to untreated fruits indicating that there is interconversion of sugars. It can be assumed that these changes in sugar and acid levels are in response to ethylene inhibition caused by NC which in turn reduced

the sugar accumulation during ripening. The role of ethylene in sugar metabolism during climacteric fruit ripening has been reported by Defilippi et al. (2004) and they verified organic acids and sugars as the ethylene-dependent metabolites by using the ethylene antagonist, 1-MCP in 'Greensleeves' apple. Kim et al. (2015) also proposed the possibility of sugar synthesis regulation by ethylene through sorbitol catabolic pathway in non-climacteric 'Sweet Miriam' plums.

5.4.6 Total phenols, ascorbic acid, antioxidant activity and anthocyanins

The amount of total phenols, ascorbic acid, anthocyanin and antioxidant capacity were slightly varied between 'Angeleno' and 'Tegan Blue' plum. Similar cultivar dependent variations in phytochemical compounds were reported in different cultivars of peaches, nectarines and plums by Diaz-Mula et al. (2009), Gil et al. (2002) and Kim et al. (2003). In 'Angeleno' plum, the levels of total phenol, ascorbic acid, anthocyanin and antioxidant capacity remained similar in both cold storage period. Similarly, Diaz-Mula et al. (2009) reported that the levels of bioactive compounds in different climacteric and suppressed-climacteric plums were not significantly affected by the cold storage at 2°C up to 35 days. In the present study, the ethylene antagonist NC did not significantly affect the levels of these compounds in both tested cultivars. This is in consistent with the findings of Defilippi et al. (2004) in Greensleeves apples where 1-MCP treated fruit retained significantly the same total phenolic level as control. The ascorbic acid contents of the fruit treated with different concentrations of 1-MCP were not different from control fruit in mango (Pauziah et al., 2014) and guava (Bassetto et al., 2005). Khan and Singh (2008) also reported that the ascorbic acid content and the total antioxidant capacity of 'Tegan Blue' plum were not affected by 1-MCP treatment. However, the levels of total phenolic content in NC treated 'Angeleno' and 'Tegan Blue' plums were comparatively lower as compared to untreated plums and this might be the consequence of reduction in sugar accumulation. In the present study, the reduction in anthocyanin concentrations of NC-treated 'Angeleno' and 'Tegan Blue' plum may be due to the influence of ethylene on anthocyanin accumulations as previously reported in blueberries and cranberry (Costa et al., 2012; Farag et al., 1992). Although many studies have been performed on profiling of the phytochemical compounds in different varieties of plum, the information on the effect of ethylene antagonist on these compounds is still limited and further investigation is warranted.

5.5 Conclusion

The ethylene antagonist NC significantly suppressed ethylene production, weight loss and fruit softening of ‘Angeleno’ and ‘Tegan Blue’ plums. The levels of SSC, SSC:TA ratio and individual sugars were maintained tendentially lower and the levels of TA and organic acids were tendentially higher in NC-treated plums. The effects of NC on these organoleptic parameters were more pronounced in fumigation treatment and in spray treatments with the presence of adjuvants either ethanol or Tween® 20. The bioactive compounds were not significantly affected by NC maintaining the same fruit quality as untreated fruit even after 40 d cold storage. In conclusion, application of NC as fumigant or as spray solutions with ethanol or with Tween® 20 can delay the ripening related physiological and quality changes through retardation of ethylene action during postharvest storage of plums.

CHAPTER 6

Effects of 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) on ethylene production and fruit quality attributes of peach, nectarine and plum

Summary

The effects of different formulations of 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) on ethylene production and fruit quality were investigated. Three separate experiments were conducted on 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum. The peach, nectarine and plum fruits were fumigated with 1µM BC or 1µM NC for 18 h or sprayed with 2µM aqueous solutions of BC and NC (containing either 5% ethanol or 0.02% Tween® 20 or both) at 20±1°C. The aqueous solutions only with either 5% ethanol, or 0.02% Tween® 20, or both ethanol and Tween® 20, were also applied to countercheck the effectiveness of BC and NC aqueous solutions. The untreated fruit were considered as control. The ethylene production and respiration rate were checked at day 0 or after 25 d storage at 1°C. The changes in other fruit physical and biochemical quality parameters were evaluated after 25 d storage at 1°C. The fumigations with BC and NC were found to be effective in suppressing and delaying the climacteric ethylene production of all the tested peach, nectarine and plum fruits in both day 0 and 25 d cold storages. The higher fruit firmness and lower fruit weight loss were also noted in the fruits fumigated with BC and NC after 25 d cold storage. The effects of aqueous solutions of BC and NC on ethylene production were varied depending on the species, the storage condition and the adjuvants applied. The respiration rate was not affected by BC and NC, regardless of formulations and the species. The effect on soluble solids content (SSC), titratable acidity (TA), SSC:TA ratio, individual sugars and organic acids, ascorbic acids, total phenols and total antioxidant capacities from BC and NC were also varied depending on the species and the formulations of BC and NC applied.

6.1 Introduction

In Australia, peach, nectarine and plum are categorised as summer fruits along with other *Prunus* species such as cherry and apricot. Western Australia is the third largest producer of peach, nectarine and plum after Victoria and New South Wales. More than 50% of the total production of peach, nectarine and plum are locally supplied for fresh

consumption and around 20% are for the fresh export market while the rest go for processing (Horticulture Innovation Australia Limited, 2017). The worldwide consumption of *Prunus* fruits has also been increasing due to their high nutritional values such as phenols, vitamins and carotenoids with the antioxidant potential (Gil et al., 2002 and 2006). However, peach, nectarine and plum are highly perishable with a limited storage life (Crisosto and Day, 2012). They tend to over ripen within one week after harvest leading to a bulk supply in the market during the fruit season. These oversupplied fruit can not only cause the reduction of the market price but huge postharvest losses, as well.

Ethylene, a gaseous natural plant hormone, is the key regulator in fruit ripening and of the subsequent biochemical changes such as fruit softening and colour development (Burg and Burg, 1965; Khan and Singh, 2008; Pech et al., 2012). The *Prunus* species are highly sensitive to ethylene and undergo a rapid fruit ripening and quality deterioration processes after harvest (Crisosto and Kader, 2000; Crisosto and Day, 2012). 1-Methylcyclopropene (1-MCP) is a well-known commercial ethylene antagonist used to postpone the ripening processes in various types of fruits by blocking the ethylene receptor and thus, the actions of ethylene (Blankenship and Dole, 2003; Watkins, 2006). 1-MCP delays the fruit ripening process by suppressing the activities of enzymes related to ethylene production and fruit softening in 'Tegan Blue' plum (Khan and Singh, 2007), in 'Yuhualu' peach (Liu et al., 2015) and in 'Maria Aurelia' nectarine (Ozkaya et al., 2016). However, there are some exceptions in the effectiveness of 1-MCP on ethylene production and fruit quality depending on the cultivar. In 'Almog' and 'Oded' peaches, the effect of 1-MCP on fruit softening and ethylene production was dependent on the concentration and the time of the treatment. The soluble solids content and respiration rate in these fruits were not affected by 1-MCP (Liguori et al., 2004).

The potential ethylene antagonists such as 1*H*-cyclopropabenzene (BC) and its derivative 1*H*-cyclopropa[*b*]naphthalene (NC) have developed recently as an alternative to 1-MCP (Singh et al., 2018). It was documented that fumigation of BC and NC can effectively delay ripening in fruits like plum and apple by blocking the ethylene receptors and the actions of ethylene in plant parts (Singh et al., 2018). There is no research work on the effectiveness of different formulations of BC and NC on ethylene production and postharvest fruit quality of peach and nectarine. The present

research was designed to investigate the effectiveness of BC and NC, either as aqueous solutions or as fumigations, on suppression of ethylene production in peach, nectarine and plum. In addition, the changes in postharvest fruit quality parameters associated with BC and NC treatments were also examined after 25 d of cold storage. It was hypothesised that the different formulations of BC and NC will delay ripening and fruit quality changes of peach, nectarine and plum by suppressing ethylene production.

6.2 Materials and methods

6.2.1 Experimental conditions

The effect of different formulations of ethylene antagonists 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[b]napathalene (NC) on ethylene production and postharvest fruit quality of peach, nectarine and plum were investigated. Three separate experiments were conducted on three different *Prunus* species: 'Princess Time' peach (*Prunus persica* L. Batsch), 'Diamond Bright' nectarine (*P. persica* L. Batsch) and 'Tegan Blue' plum (*P. salicina* L). The detailed information on the source of fruits, the ethylene antagonist applied and the experimental site is described in Chapter 3, section 3.3.

In the first experiment, 'Princess Time' peach fruit were collected at commercial harvest stage (11.7±0.1% TSS, 2.5±0.0% TA and 38±1.5 N firmness) from the Eastwind orchard on 8th December 2017. The total 648 fruit free from any physiological defects were selected as the fruit sample. The selected fruit were divided into 12 lots. The fruit were then subjected to eleven different treatments (as shown in table 6.1) and the untreated lot was considered as control. The treatments 1 (ethanol (5%) aqueous solution), 2 (Tween® 20 (0.02%) aqueous solution) and 3 (ethanol (5%) + Tween® 20 (0.02%) aqueous solution) were applied in order to countercheck the effectiveness of BC and NC aqueous solutions. The experiment was carried out using a completely randomised design with three replications. The preparation of BC and NC spray solutions and the application of the treatments were described in Section 3.3. After applying the respective treatments, the treated and untreated fruit were stored for 25 d at 1°C and 90±5 % RH. At the end of the cold storage period, the fruit were taken out to room temperature (20±1 °C and 85±5 % RH) in order to check the fruit

physiological and biochemical quality parameters. Ethylene production and respiration rate were checked at before (0 d cold storage) or after 25 d storage at 1 °C.

The second experiment was conducted on 'Diamond Bright' nectarine fruit (9.6±0.4 % TSS, 2.1±0.1 % TA and 41±2.1 N firmness) on 21st December 2017 following the same experimental condition and procedure as in experiment 1.

The third experiment was conducted on 'Tegan Blue' plum fruit (11.7±0.1 % TSS, 2.5±0.0 % TA and 38±1.5 N firmness) on 9th February 2018 following the same experimental condition and procedure as in experiment 1.

Table 6. 1 The different formulation treatments of BC and NC applied.

No.	Treatments
1.	ethanol (5%) aqueous solution
2.	Tween® 20 (0.02%) aqueous solution
3.	ethanol (5%) + Tween® 20(0.02%) aqueous solution
4.	BC (2 µM)+ ethanol (5%) aqueous solution
5.	BC (2 µM)+ Tween® 20 (0.02%) aqueous solution
6.	BC (2 µM)+ ethanol (5%) plus Tween® 20(0.02%) aqueous solution
7.	BC (1 µM) fumigation
8.	NC (2 µM)+ ethanol (5%) aqueous solution
9.	NC (2 µM)+ Tween® 20 (0.02%) aqueous solution
10.	NC (2 µM)+ ethanol (5%) plus Tween® 20 (0.02%) aqueous solution
11.	NC (1 µM) fumigation
12.	Without any treatment (control)

6.2.2 Determination of physiological parameters

6.2.2.1 Ethylene production (nmol kg^{-1} h^{-1} and µmol kg^{-1} h^{-1})

Ethylene production of peach, nectarine and plum was checked using three representative fruit per replication by following the procedure described by Khan and Singh (2008) and Azzu (2016). The determination of ethylene production for peach and nectarine was done by using a gas chromatogram (GC) and for plum by an ETD

300 sensor sense. The detailed information on the determination of ethylene production is described in Chapter 3, section 3.5.1.

6.2.2.2 Respiration rate (mmol CO_2 kg^{-1} h^{-1})

The same sample fruit selected to check ethylene production were used for the determination of the respiration rate. The respiration rate (CO_2 production rate) was determined by following the method described by Khan and Singh (2008) as previously explained in Chapter 3, section 3.5.2

6.2.2.3 Physiological weight loss (%) and fruit firmness (N)

Physiological weight loss was determined following the procedure described by Azzu (2016) as mentioned in Chapter 3, section 3.5.3.

Fruit firmness (N) was determined by using a texture analyser fitted with 8 mm probe following the method previously described by Khan and Singh (2008). The detailed procedure for the determination of fruit firmness is reported in Chapter 3, section 3.5.4.

6.2.3 Determination of quality parameters

6.2.3.1 SSC (%), TA (%) and SSC:TA

The soluble solids content, titratable acidity and their ratio were determined by following the procedure described by Khan and Singh (2008). Sample preparation and quantification of SSC, TA and SSC:TA is detailed in Chapter 3, section 3.6.1.

6.2.3.2 Individual sugars and organic acids (g kg^{-1})

Individual sugars and organic acids were determined by using a high-performance liquid chromatography (HPLC) following the procedure described by Usenik et al. (2008) with some modifications. The individual sugars (glucose, fructose, sucrose and sorbitol) and the individual organic acids (malic, citric, fumaric and succinic acids) were identified by standardising with the respective standard sugars and acids. The procedures for sample preparation and quantification are described in Chapter 3, section 3.6.2.

6.2.3.3 Total phenols (g kg^{-1})

Total phenols were quantified following the procedure described by Cantin et al. (2009) with little modifications. The procedures for extraction of total phenols and the

protocol for quantification of the total phenolic content are described in Chapter 3, section 3.6.3.

6.2.3.4 Ascorbic acid (mg kg^{-1})

The ascorbic acid content was quantified following the procedure described by Vithana (2017). The detailed procedure for the extraction and the assessment of ascorbic acid concentration is mentioned in Chapter 3, section 3.6.4.

6.2.3.5 Total antioxidant capacity (mmol Trolox kg^{-1})

The total antioxidant capacity was estimated by checking the free radical scavenging capacity using the DPPH method based on Brand-Williams et al. (1995) as previously described by Vithana (2017). The procedures for the preparation of standard DPPH solution, the extraction and quantification of antioxidant are described in Chapter 3, section 3.6.5.

6.2.4 Statistical analysis

The recorded data were analysed by using Genstat software (13^{th} edition), Genstat release 13.1, VSN International Ltd., UK. The data for peach, nectarine and plum were analysed separately. The least significant differences (LSD) at ($P \leq 0.05$) were checked to compare the treatment effects. The data were described as mean ± standard error of the mean. Duncan's multiple range test was used to compare the treatment means.

6.3 Results

6.3.1 Ethylene production

Ethylene production of 'Princess Time' peach fumigated with BC and NC was tendentially suppressed as compared to control after 0 d and 25 d cold storage (Fig. 6.1 A and B). Among the aqueous solution treatments, only NC aqueous solution with ethanol significantly suppressed ethylene production as compared to control and its countercheck-treatment (ethanol alone aqueous solution) after 0 and 25 d cold storage (Fig. 6.1 A and B). The onsets of climacteric ethylene peak in 'Princess Time' (PT) peach treated with NC aqueous solution with Tween® 20 was significantly delayed ≈ 2 days as compared to control after 25 d cold storage (Table 6.3). The ethylene climacteric peak concentrations of the fruit fumigated with NC were also significantly reduced 1.6 and 2.0 fold, respectively as compared to control after 0 and 25 d cold

storages (Table 6.2 and 6.3). The NC aqueous solution with ethanol significantly reduced (1.5 fold each) climacteric ethylene peak concentration as compared to control and the ethanol alone aqueous solution after 25 d cold storage (Table 6.3).

The ethylene production of 'Diamond Bright' nectarine fumigated with BC and NC, and treated with NC aqueous solutions were tendentially suppressed as compared to control after 0 and 25 d cold storages (Fig. 6.2 A and B). The BC aqueous solutions with Tween® 20 and with ethanol solutions significantly reduced ethylene production of DB nectarine as compared to control and the counter treatments after 0 d cold storage (Fig. 6.2 A). The onset of climacteric ethylene peak were significantly delayed (2 d each) in the fruit fumigated with BC and NC after 0 d cold storage (Table 6.2). The climacteric ethylene concentrations of the fruit treated with NC fumigation were significantly reduced (1.6 and 4.9 fold, respectively) as compared to control after 0 d and 25 d cold storage (Table 6.2 and 6.3).

The ethylene production of 'Tegan Blue' plum was significantly suppressed by BC and NC, irrespective of the formulations, as compared to control after 0 d cold storage (Fig. 6.3 A). After 25 d cold storage, the ethylene production of the fruit treated with fumigations and aqueous solutions, except BC and NC solutions with Tween® 20, were significantly reduced as compared to control (Fig. 6.3 B). The onset of climacteric ethylene peak in the fruit treated with BC and NC fumigations, BC and NC aqueous solutions with ethanol and Tween® 20were significantly delayed as compared to control after 0 d cold storage (Table 6.2). The climacteric ethylene concentration of the fruit treated with BC and NC, regardless of formulations, were significantly lower than control after 0 d cold storage (Table 6.2). The lowest peak concentration (0.3 nmol $kg^{-1}h^{-1}$) was observed in the fruit fumigated with NC. After 25 d cold storage, the fruit treated with NC fumigation, NC aqueous solutions with ethanol, with ethanol and Tween® 20, and BC aqueous solution with ethanol, exhibited the significantly suppressed climacteric ethylene peak concentration as compared to control (Table 6.3). The NC treated fruit, except NC aqueous solution with Tween® 20, showed the significantly lowest amount of ethylene production, which were 2 fold each lower than control after 25 d cold storage (Table 6.3).

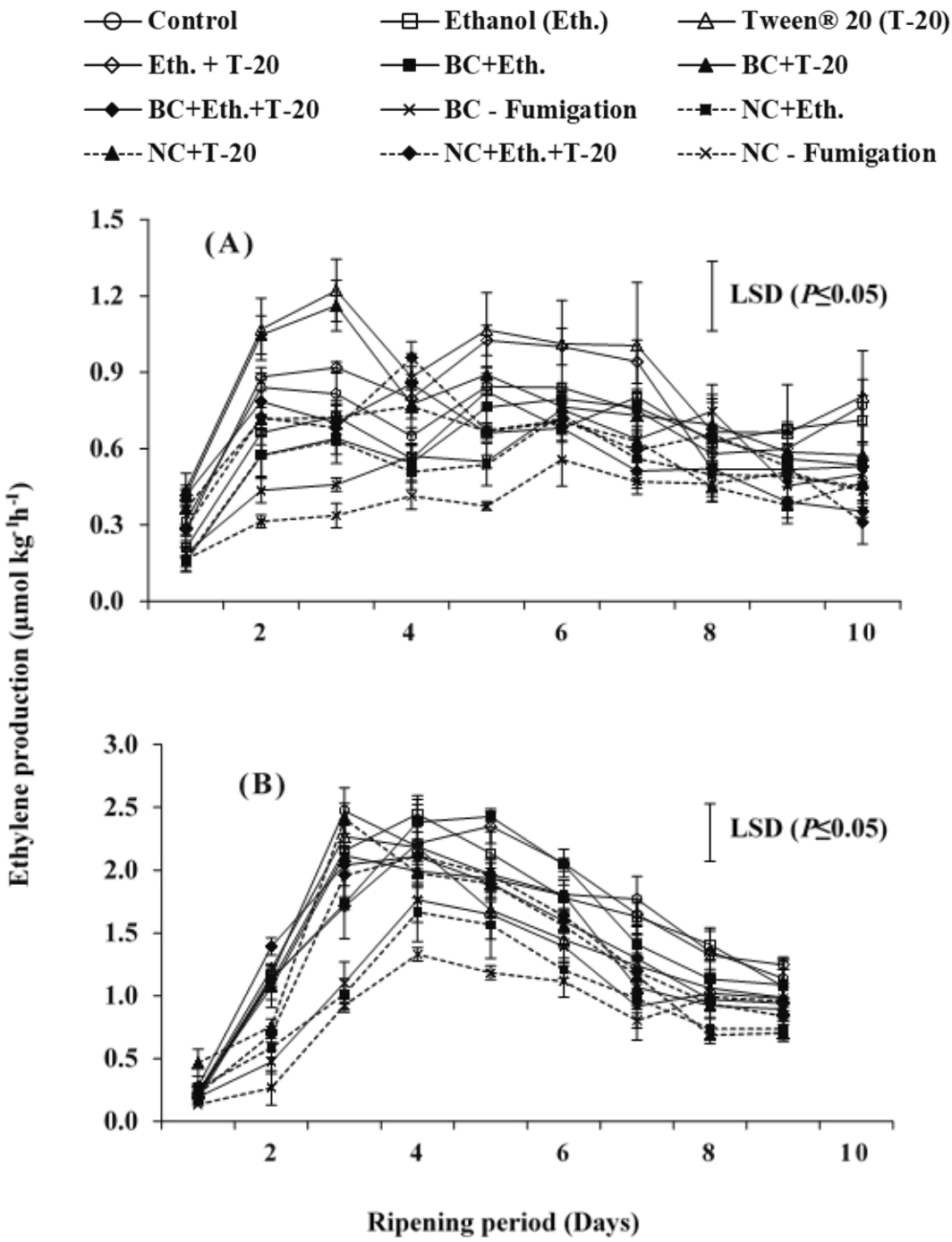

Fig. 6. 1 Effect of different formulations (as aqueous solutions and fumigant) of BC and NC on ethylene production of 'Princess Time' peach (A) under ambient condition and (B) after 25 d cold storage. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for ethylene production of (A): treatments (tr) =0.09, days after storage (d) =0.08 and their interaction (tr x d) =0.27, and (B): tr=0.17, d=0.15 and tr x d= 0.5.

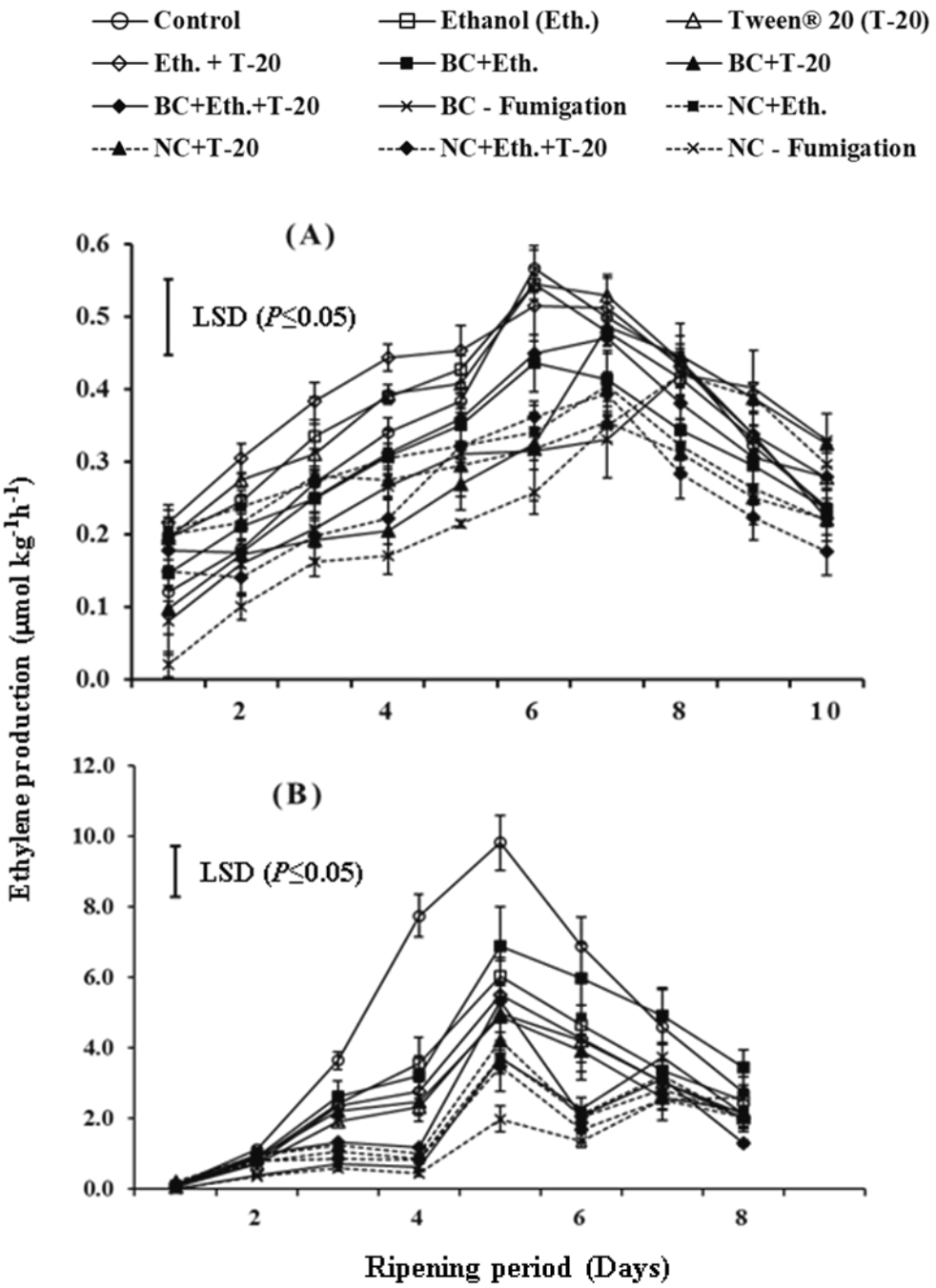

Fig. 6. 2 Effect of different formulations (as aqueous solutions and fumigant) of BC and NC on ethylene production of 'Diamond Bright' nectarine (A) under ambient condition and (B) after 25 d cold storage. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for ethylene production of (A): treatments (tr) =0.03, days after storage (d) =0.03 and their interaction (tr x d) =0.11, and (B): tr=0.47, d=0.38 and tr x d= 1.3.

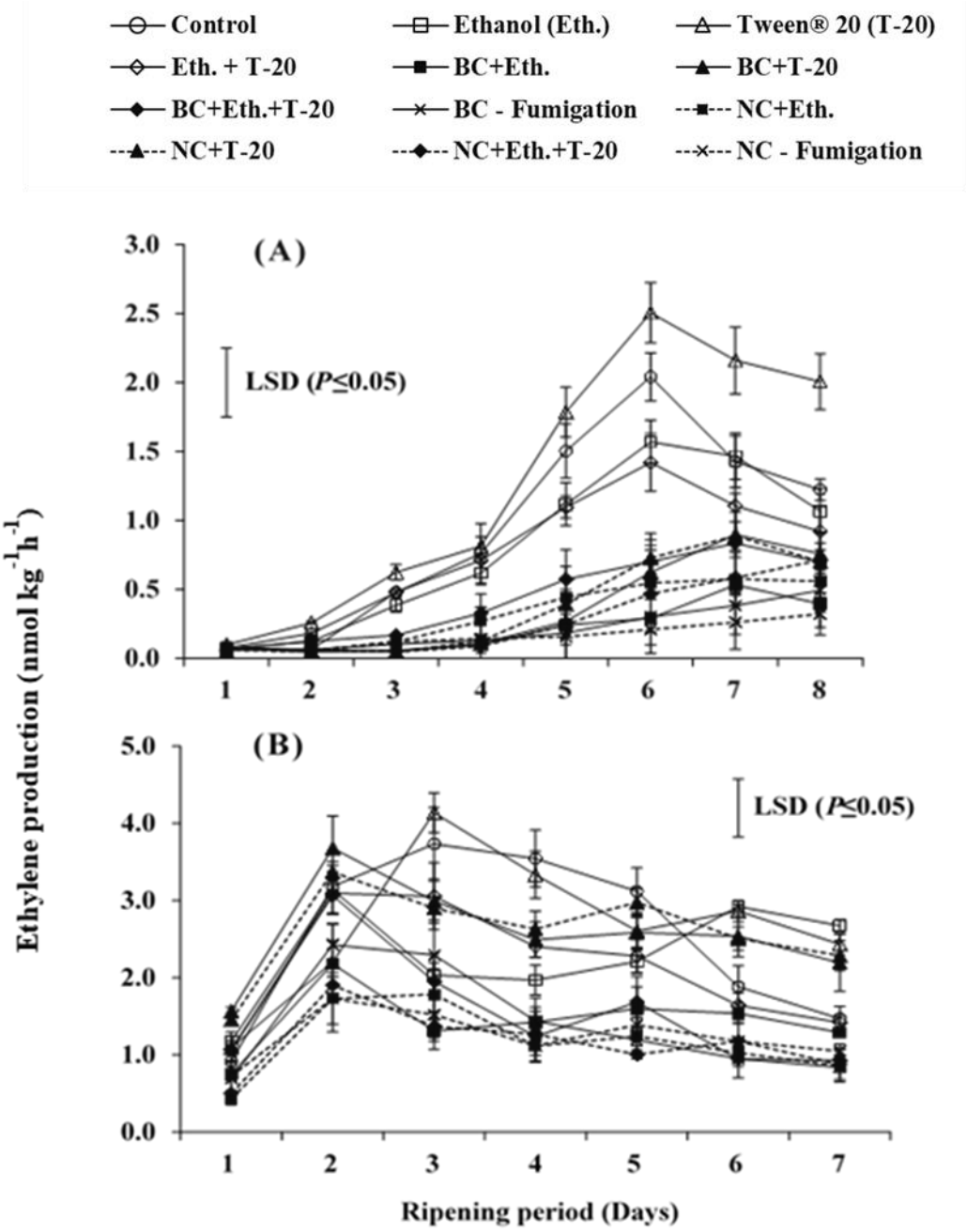

Fig. 6. 3 Effect of different formulations (as aqueous solutions and fumigant) of BC and NC on ethylene production of 'Tegan Blue' plum (A) under ambient condition and (B) after 25 d cold storage. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for ethylene production of (A): treatments (tr) =0.09, days after storage (d) =0.07 and their interaction (tr x d) =0.24, and (B): tr=0.31, d=0.23 and tr x d= 81.

Table 6. 2 The climacteric ethylene peak onset and the climacteric ethylene peak rate of 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum affected by different formulations of ethylene antagonists BC and NC after 0 d cold storage.

Treatments	Climacteric ethylene peak onset			Climacteric ethylene peak concentration		
	Princess Time (d)	Diamond Bright (d)	Tegan Blue (d)	Princess Time (μmol $kg^{-1}h^{-1}$)	Diamond Bright (μmol $kg^{-1}h^{-1}$)	Tegan Blue (nmol $kg^{-1}h^{-1}$)
Control	3.7±1.4	6.0±0.0a	6.0±0.0ab	0.9±0.0bcd	0.6±0.0c	2.0±0.1f
Ethanol(Eth.)	4.3±0.5	6.0±0.0a	6.3±0.3abc	0.8±0.1abc	0.6±0.0c	1.7±0.1e
Tween® 20(T-20)	3.0±0.0	6.0±0.0a	6.3±0.3abc	1.2±0.1e	0.6±0.0c	2.6±0.2g
Eth. +T-20	3.0±0.0	6.3±0.3ab	5.7±0.3a	1.0±0.1cde	0.5±0.1bc	1.5±0.1e
BC + Eth.	6.0±0.5	6.7±0.3ab	7.0±0.0cde	0.8±0.1abc	0.4±0.1abc	0.5±0.0abc
BC + T-20	5.3±0.3	7.3±0.3b	7.3±0.3def	1.2±0.1de	0.5±0.0abc	0.9±0.1d
BC+ Eth. + T-20	4.7±0.5	6.7±0.3ab	7.3±0.3def	0.9±0.1abc	0.5±0.0abc	0.9±0.0cd
BC-Fumigation	6.0±0.0	8.3±0.3c	8.0±0.0f	0.8±0.0abc	0.4±0.0abc	0.5±0.1ab
NC + Eth.	6.0±0.0	7.0±0.0ab	7.0±0.5cde	0.7±0.1ab	0.4±0.0ab	0.6±0.1abcd
NC + T-20	4.7±0.5	7.0±0.5ab	6.7±0.3bcd	0.9±0.0bcd	0.43±0.0abc	0.9±0.0cd
NC+ Eth. + T-20	5.3±1.1	6.3±0.3ab	7.7±0.3ef	1.0±0.0cde	0.4±0.0abc	0.8±0.1bcd
NC-Fumigation	5.3±0.5	8.3±0.3c	8.0±0.0f	0.6±0.1a	0.37±0.0a	0.3±0.1a
LSD ($P \leq 0.05$)	ns	0.9**	0.75**	0.25**	0.12*	0.34**

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 6. 3 The climacteric ethylene peak onset and the climacteric ethylene peak rate of ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum affected by different formulations of ethylene antagonists BC and NC after 25 d cold storage.

Treatments	Climacteric ethylene peak onset			Climacteric ethylene peak concentration		
	Princess Time (d)	Diamond Bright (d)	Tegan Blue (d)	Princess Time (μmol $kg^{-1}h^{-1}$)	Diamond Bright (μmol $kg^{-1}h^{-1}$)	Tegan Blue (nmol $kg^{-1}h^{-1}$)
Control	3.3±0.3ab	5.0±0.0	2.0±0.0	3.1±0.1e	9.8±0.5d	3.7±0.3cd
Ethanol(Eth.)	3.7±0.3abc	5.0±0.0	2.0±0.0	2.5±0.1cde	6.0±0.5bc	3.4±0.2bcd
Tween® 20(T-20)	3.3±0.3ab	5.0±0.0	2.3±0.3	2.3±0.2bcd	5.3±0.5bc	4.1±0.5d
Eth. +T-20	3.0±0.0a	5.0±0.0	2.3±0.3	2.5±0.2cde	5.5±1.1bc	3.7±0.3cd
BC + Eth.	4.7±0.3c	5.0±0.0	2.0±0.0	2.5±0.1de	6.9±1.1c	2.1±0.2a
BC + T-20	3.3±0.3ab	5.0±0.0	2.0±0.0	2.2±0.2bcd	4.9±0.5bc	3.3±0.3bcd
BC+ Eth. + T-20	3.7±0.3ab	5.0±0.0	2.3±0.3	2.1±0.1abcd	4.9±0.5bc	3.1±0.3abcd
BC-Fumigation	4.3±0.3bc	5.0±0.0	3.3±1.1	1.8±0.1abc	3.7±0.4ab	2.5±0.5abc
NC + Eth.	4.3±0.3bc	5.0±0.0	2.3±0.3	1.7±0.2ab	3.8±0.4ab	1.9±0.4a
NC + T-20	4.7±0.3c	5.0±0.0	3.0±0.0	2.4±0.1cd	4.2±0.6abc	3.4±0.4bcd
NC+ Eth. + T-20	3.7±0.3abc	5.0±0.0	3.0±0.0	2.2±0.1bcd	3.5±0.7ab	1.9±0.2a
NC-Fumigation	4.3±0.3bc	5.0±0.0	3.0±0.8	1.5±0.3a	2.0±0.4a	1.9±0.2a
LSD ($P \leq 0.05$)	0.97*	ns	ns	0.58**	2.4**	1.2*

Means in the same column followed by the different letters are significantly different by Duncan’s multiple range test at $P \leq 0.05$. The letterings for ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

6.3.2 Respiration rate

The respiration rates of 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum treated with different formulations of BC and NC were not significantly different to the control, except in 'Princess Time' peach after 0 d cold storage (Fig. 6.4, 6.5 and 6.6). After 0 d cold storage, the peach treated with different formulations of BC and NC exhibited significantly lower respiration rate till the 8^{th} day of ripening period as compared to the control fruit (Fig. 6.4 A). However, the peach treated with different BC and NC aqueous solutions were not significantly different from their countercheck-treatments (Fig. 6.4 A).

The onsets of climacteric respiration peak in all the treated fruit, except 'Tegan Blue' after 0 d cold storage and 'Princess Time' peach after 25 d cold storage, were observed on the same day as that of untreated fruit (Table 6.4 and 6.5). After 0 d cold storage, the commencements of climacteric respiration peak in 'Tegan Blue' plum treated with BC fumigation, NC aqueous solutions with Tween® 20 alone, and with ethanol and Tween® 20, were one day later compared to control and their countercheck-treatments (Table 6.4). Similarly, 'Princess Time' peach treated with BC fumigation and NC aqueous solution with ethanol also exhibited the respiration peak onset one day later than control and the ethanol alone aqueous solution after 25 d cold storage (Table 6.5). None of the BC and NC formulations showed any significant effect on the climacteric respiration peak rates of all the tested cultivars after 0 and 25 d cold storage (Table 6.4 and 6.5).

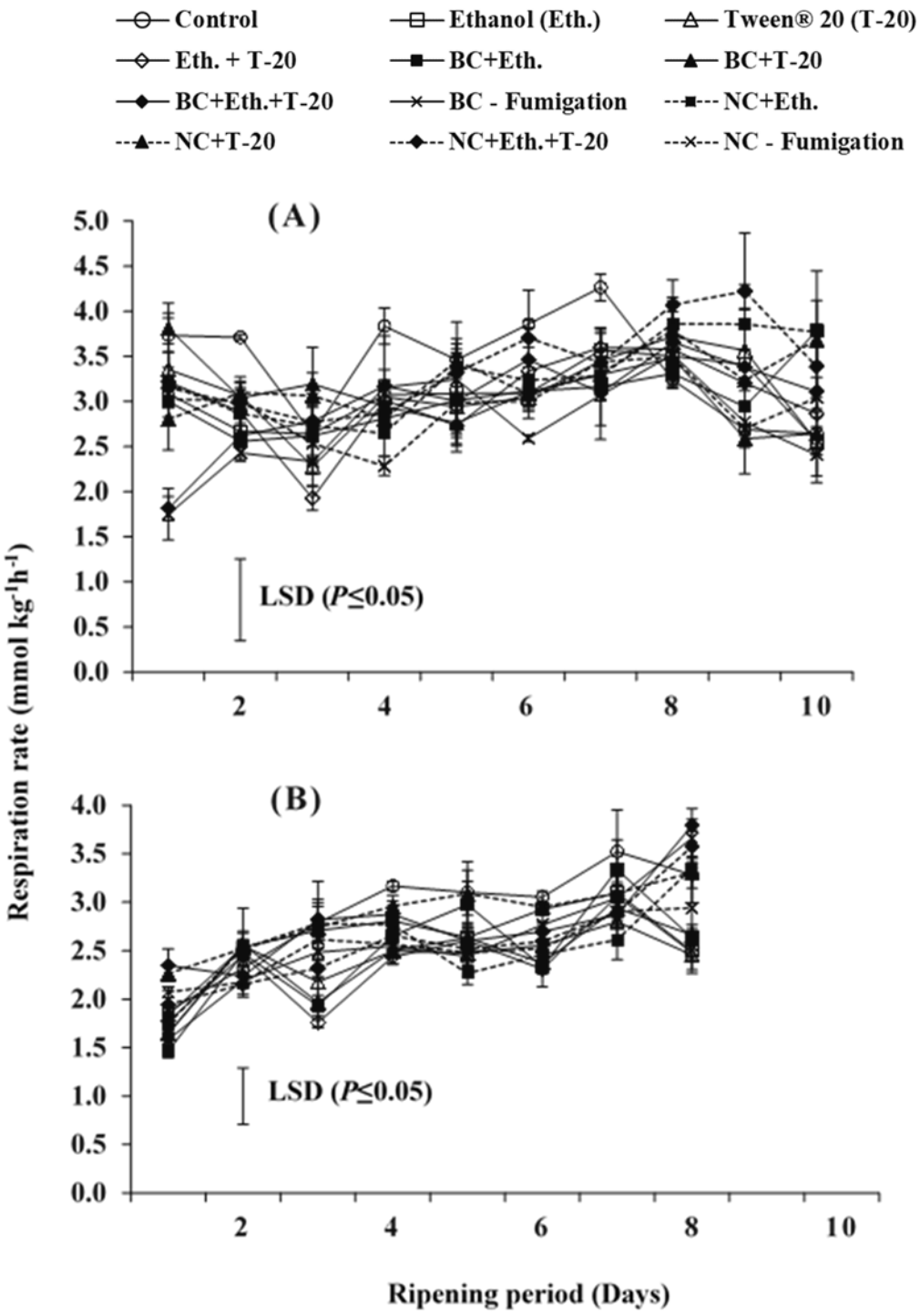

Fig. 6. 4 Effect of different formulations (as aqueous solutions and fumigant) of BC and NC on the respiration rate of 'Princess Time' peach (A) under ambient condition and (B) after 25-d cold storage. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for respiration rate of (A): treatments (tr) =0.33, days after storage (d) =0.30 and their interaction (tr x d) =ns, and (B): tr=0.19, d=0.16 and tr x d= 55.

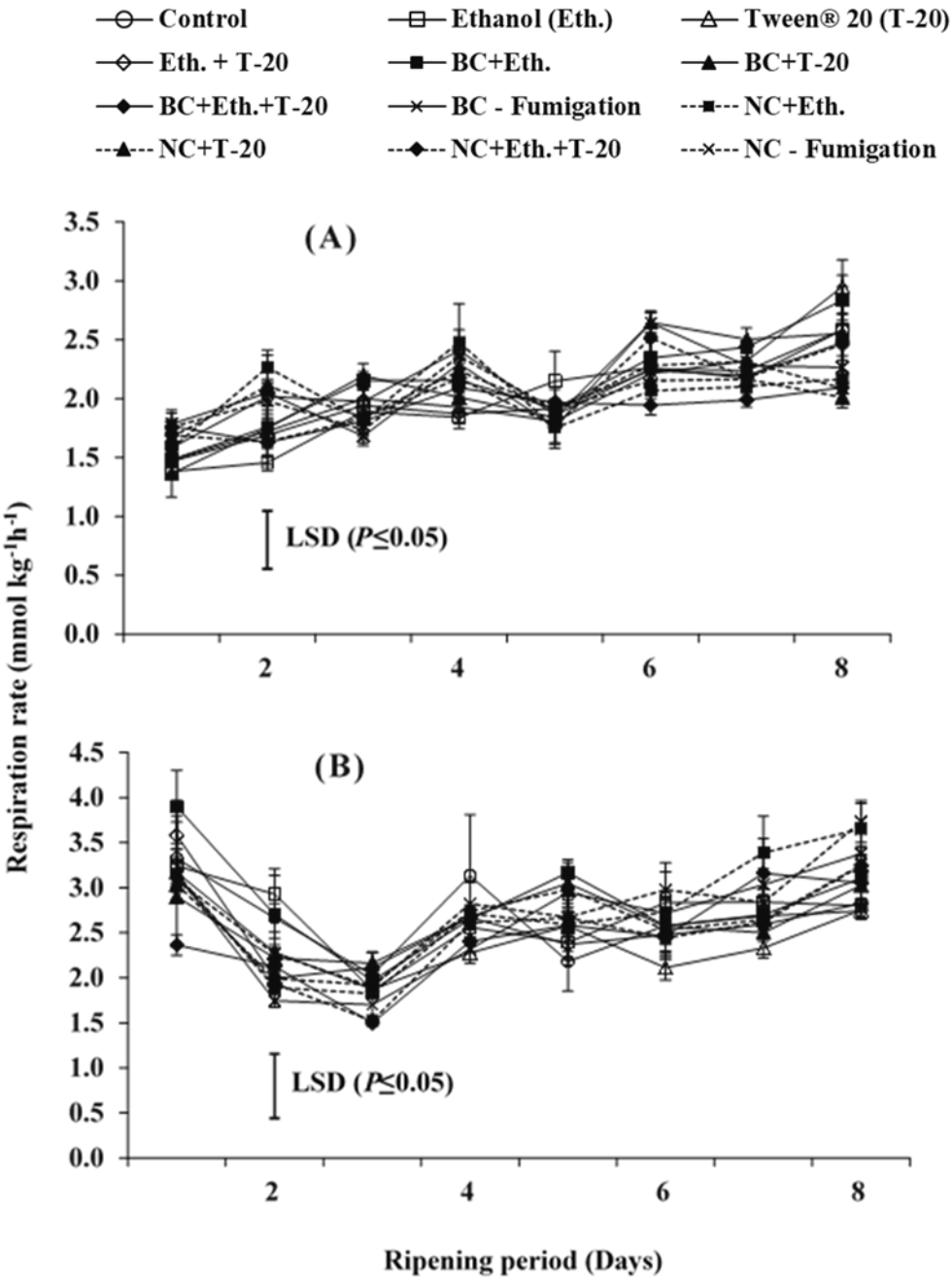

Fig. 6. 5 Effect of different formulations (as aqueous solutions and fumigant) of BC and NC on the respiration rate of 'Diamond Bright' nectarine (A) under ambient condition and (B) after 25 d cold storage. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for respiration rate of (A): treatments (tr) =0.18, days after storage (d) =0.16 and their interaction (tr x d) =0.55, and (B): tr=ns, d=0.25 and tr x d= ns.

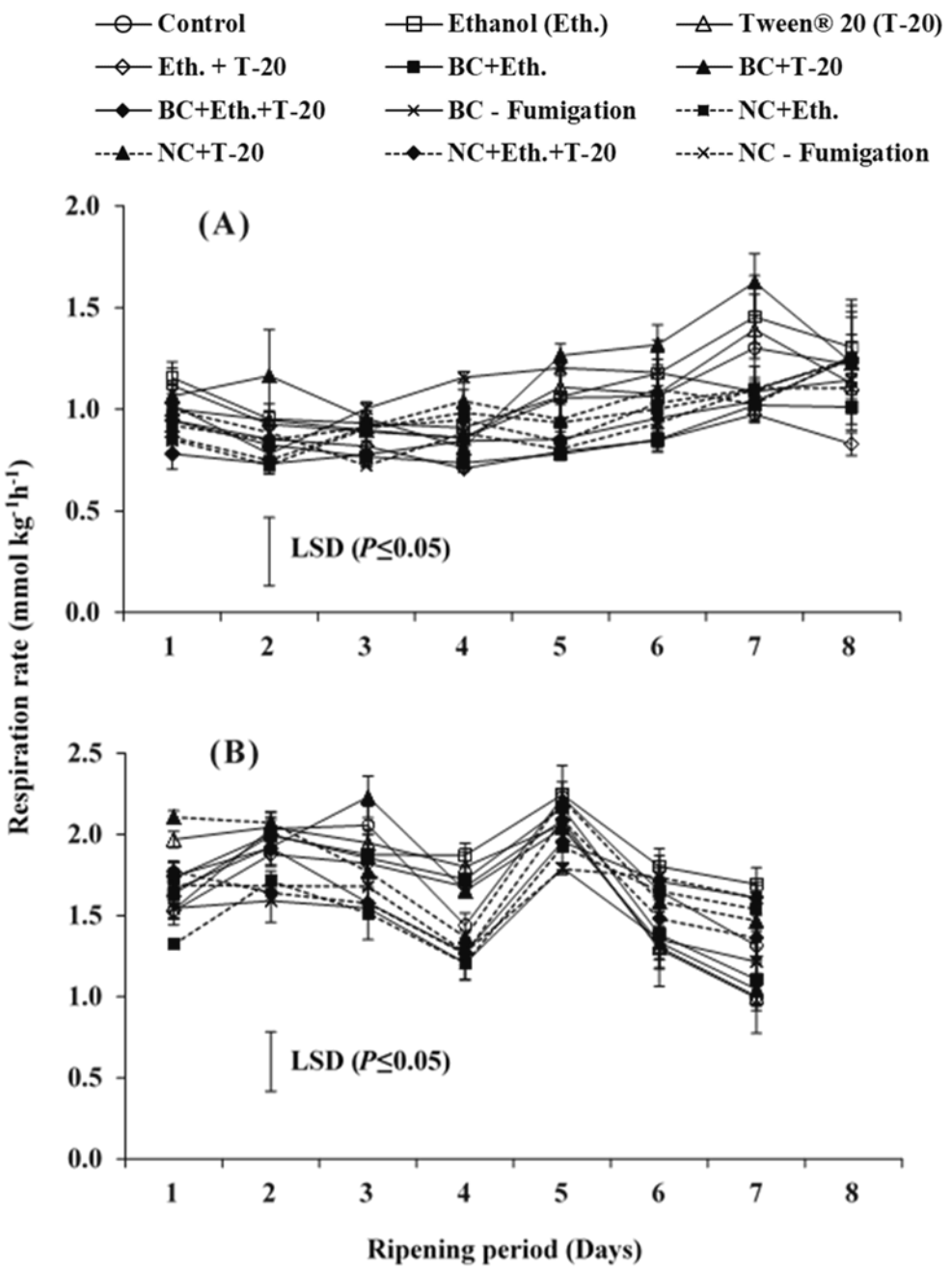

Fig. 6. 6 Effect of different formulations (as aqueous solutions and fumigant) of BC and NC on the respiration rate of 'Tegan Blue' plum (A) under ambient condition and (B) after 25 d cold storage. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for respiration rate of (A): treatments (tr) =0.12, days after storage (d) =0.1 and their interaction (tr x d) =ns, and (B): tr=0.14, d=0.11 and tr x d= 37.

Table 6. 4 The climacteric respiration peak onset and the climacteric respiration peak rate of ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum affected by different formulations of ethylene antagonists BC and NC after 0 d cold storage.

Treatments	Climacteric respiration peak onset			Climacteric respiration peak rate		
	Princess Time (d)	Diamond Bright (d)	Tegan Blue (d)	Princess Time (μmol kg^{-1} h^{-1})	Diamond Bright (μmol kg^{-1} h^{-1})	Tegan Blue (nmol kg^{-1} h^{-1})
Control	6.3±1.4	4.7±0.5	7.0±0.0abc	5.2±0.6	2.6±0.2	1.3±0.3
Ethanol(Eth.)	6.3±0.5	5.3±0.5	7.0±0.0abc	3.8±0.3	2.3±0.2	1.5±0.2
Tween® 20(T-20)	7.0±0.8	6.0±1.0	7.0±0.0abcd	4.3±0.5	2.5±0.2	1.4±0.1
Eth. +T-20	7.0±0.5	5.7±0.3	6.7±0.3a	3.7±0.3	2.2±0.1	1.0±0.0
BC + Eth.	5.7±1.2	4.3±0.7	7.3±0.3abcde	3.6±0.2	3.2±0.5	1.1±0.1
BC + T-20	6.3±0.9	5.0±0.8	7.0±0.0ab	3.8±0.2	2.6±0.1	1.6±0.1
BC+ Eth. + T-20	6.3±0.7	4.3±0.3	7.7±0.3bcde	4.0±0.2	2.1±0.1	1.2±0.1
BC-Fumigation	4.7±0.3	6.3±0.3	8.0±0.0e	3.9±0.4	2.7±0.1	1.9±0.1
NC + Eth.	6.0±0.8	5.3±0.5	7.7±0.3bcde	3.7±0.2	2.4±0.2	1.3±0.2
NC + T-20	7.3±0.9	5.7±0.7	8.0±0.0e	4.0±0.3	2.4±0.1	1.3±0.1
NC+ Eth. + T-20	5.7±1.2	5.7±0.7	8.0±0.0e	4.0±0.2	2.6±0.2	1.2±0.0
NC-Fumigation	7.3±0.3	5.7±0.7	7.7±0.3bcde	3.7±0.2	2.5±0.2	1.2±0.1
LSD ($P \leq 0.05$)	ns	ns	0.6**	ns	ns	ns

Means in the same column followed by the different letters are significantly different by Duncan’s multiple range test at $P \leq 0.05$. The letterings for ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 6. 5 The climacteric respiration peak onset and the climacteric respiration peak rate of ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum affected by different formulations of ethylene antagonists BC and NC after 25 d cold storage.

Treatments	Climacteric respiration peak onset			Climacteric respiration peak rate		
	Princess Time (d)	Diamond Bright (d)	Tegan Blue (d)	Princess Time (μmol $kg^{-1}h^{-1}$)	Diamond Bright (μmol $kg^{-1}h^{-1}$)	Tegan Blue (nmol $kg^{-1}h^{-1}$)
Control	6.7±0.3ab	3.7±0.7	4.3±0.5	3.0±0.1	3.3±0.6	2.3±0.1
Ethanol(Eth.)	6.7±0.3ab	5.3±0.5	5.0±0.0	3.2±0.2	3.1±0.3	2.2±0.2
Tween® 20(T-20)	6.3±0.5a	5.7±0.5	4.3±0.5	2.9±0.2	2.6±0.3	2.1±0.1
Eth. +T-20	7.0±0.0abc	4.3±0.3	5.0±0.0	3.1±0.1	2.8±0.1	2.1±0.1
BC + Eth.	7.0±0.0abc	4.7±0.3	5.0±0.0	3.4±0.3	3.2±0.2	2.2±0.1
BC + T-20	6.7±0.3ab	5.0±0.0	5.0±0.0	2.9±0.2	3.1±0.2	2.0±0.1
BC+ Eth. + T-20	8.0±0.0c	4.7±0.3	5.0±0.0	3.8±0.1	2.6±0.1	1.9±0.1
BC-Fumigation	8.0±0.0c	5.0±0.0	5.0±0.0	3.7±0.3	3.6±0.7	1.8±0.0
NC + Eth.	8.0±0.0c	5.0±0.5	5.3±0.3	3.4±0.1	2.8±0.1	1.9±0.1
NC + T-20	7.0±0.8abc	4.7±0.3	5.0±0.0	3.6±0.1	3.1±0.2	2.2±0.1
NC+ Eth. + T-20	8.0±0.0c	4.7±0.3	5.0±0.0	3.6±0.2	2.7±0.1	2.1±0.1
NC-Fumigation	7.7±0.3bc	4.3±0.3	5.3±0.3	3.9±0.3	3.7±0.6	1.9±0.1
LSD ($P \leq 0.05$)	1.1*	ns	ns	ns	ns	ns

Means in the same column followed by the different letters are significantly different by Duncan’s multiple range test at $P \leq 0.05$. The letterings for ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

6.3.3 Physiological weight loss and fruit firmness

Physiological weight loss of ‘Diamond Bright’ nectarine fumigated with BC and NC and ‘Tegan Blue’ plum fumigated with BC were reduced up to 1.5 and 1.2 fold respectively as compared to control after 25 d cold storage (Fig. 6.7 B and C). The weight loss of ‘Diamond Bright’ nectarine treated with BC aqueous solutions with ethanol alone, with Tween® 20 alone, and NC aqueous solutions with ethanol and Tween® 20, were significantly lower up to 1.4 fold than control but not different from their respective countercheck aqueous solution treatments (Fig. 6.7 B). No significant difference was observed between the treated and untreated ‘Princess Time’ peaches and ‘Tegan Blue’ plums after 25 d cold storage (Fig. 6.7 A).

Fumigation with BC and NC significantly maintained higher fruit firmness in ‘Princess Time’ peach and ‘Diamond Bright’ nectarine ‘Tegan Blue’ plums as compared to control (Fig. 6.8). The fruit firmness of all the cultivars treated with BC and NC aqueous solutions, except BC solution with ethanol alone, NC solution with ethanol alone, and NC solution with ethanol and Tween® 20, were not different from controls. The BC aqueous solution with ethanol retained 1.4 fold higher fruit firmness of ‘Tegan Blue’ plum than control and the ethanol alone aqueous solution. The fruit firmness of ‘Princess Time’ peach treated with NC solution containing ethanol and Tween® 20 and ‘Tegan Blue’ plum treated with NC solution containing ethanol were significantly lower 1.2 and 1.4 fold than the respective controls, but not significantly different from them.

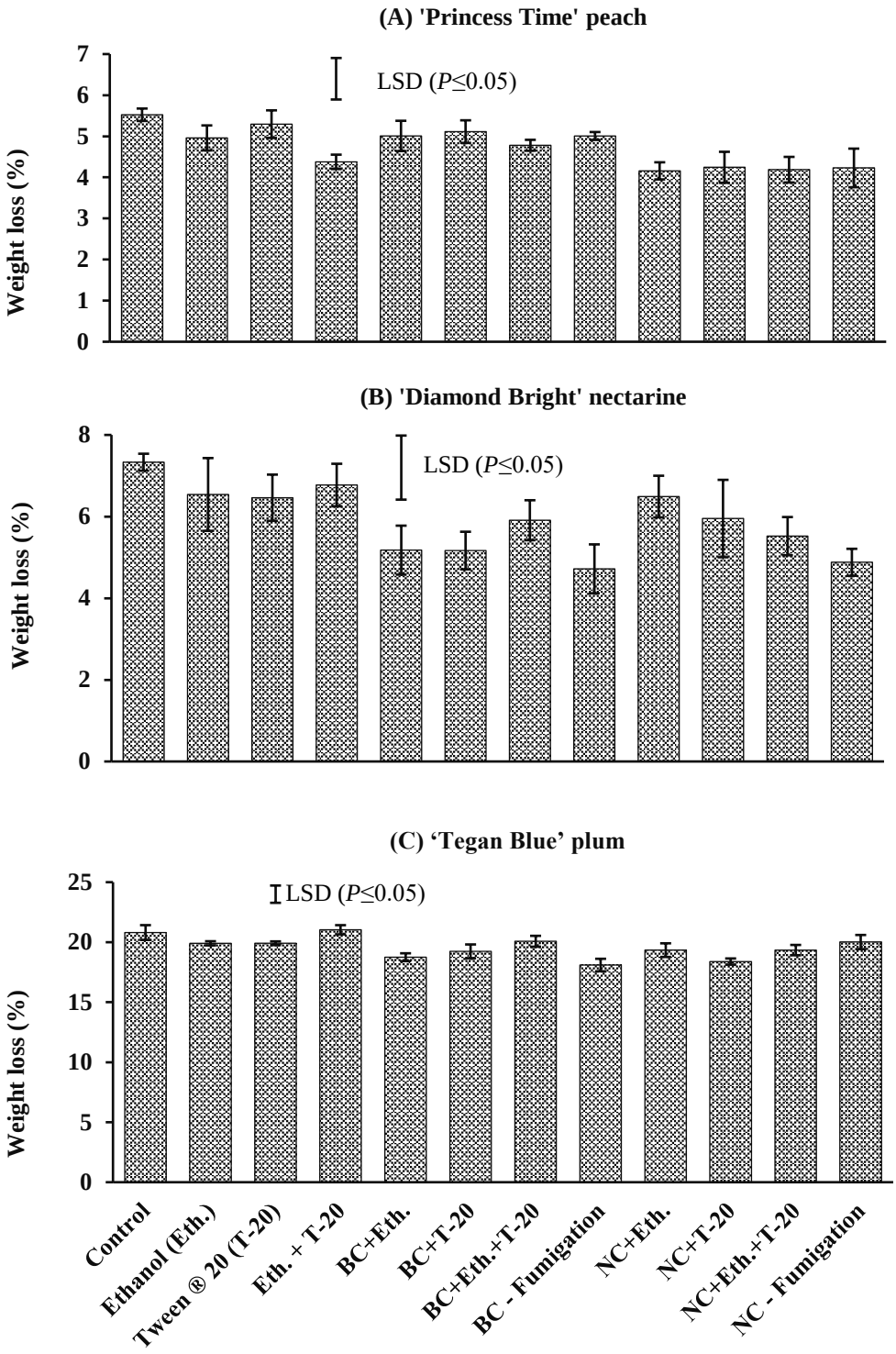

Fig. 6. 7 Effect of different formulations (as aqueous solutions and fumigant) of BC and NC on weight loss of 'Princess Time' peach (A), 'Diamond Bright' nectarine (B) and 'Tegan Blue' plum (C) under ambient condition after 25 d cold storage. Vertical bars represent SE of means of three replicates and are not visible when the values are too small.

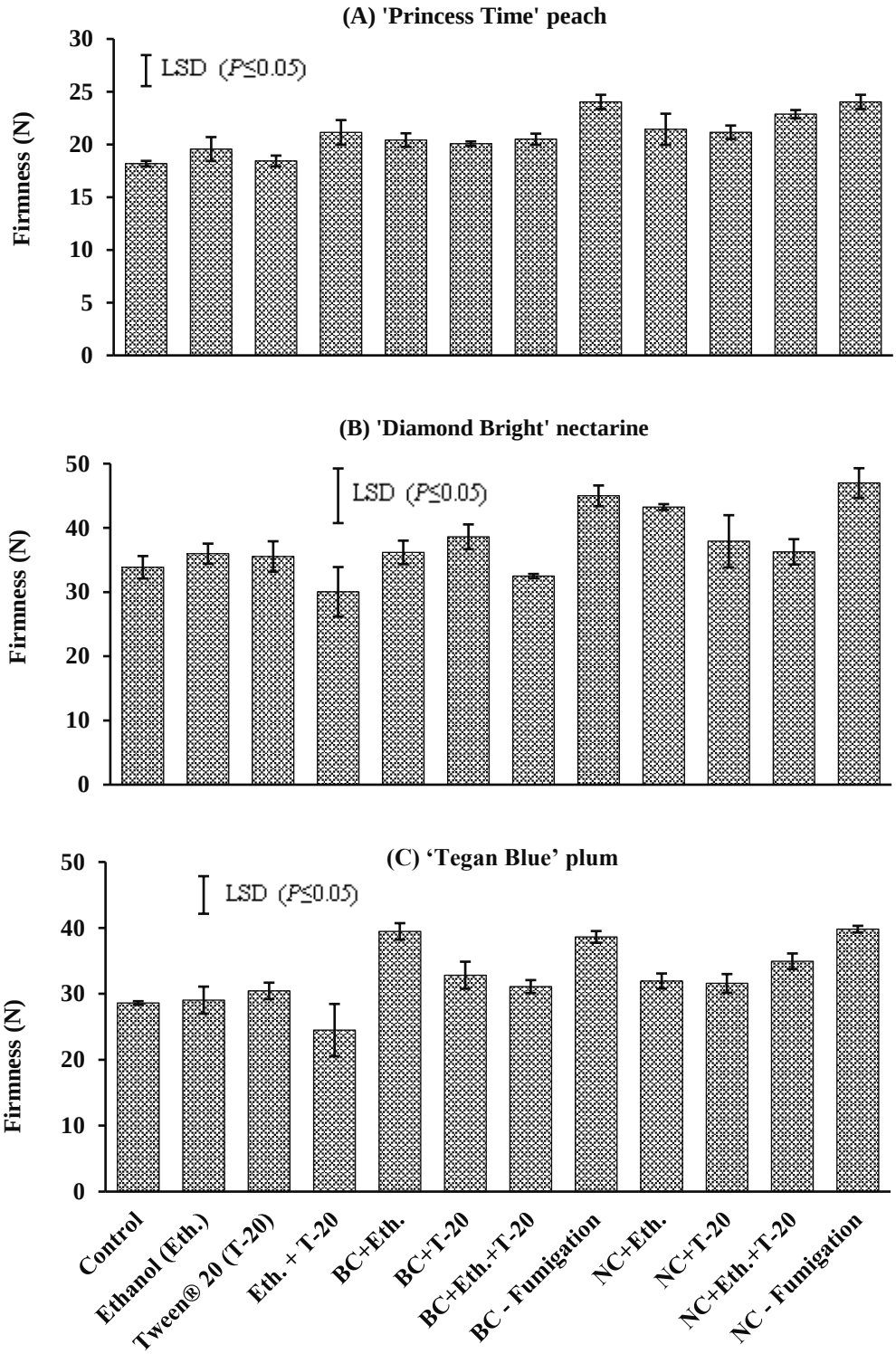

Fig. 6. 8 Effect of different formulations (as aqueous solutions and fumigant) of BC and NC on firmness of 'Princess Time' peach (A), 'Diamond Bright' nectarine (B) and 'Tegan Blue' plum (C) under ambient condition after 25 d cold storage. Vertical bars represent SE of means of three replicates and are not visible when the values are too small.

6.3.4 SSC, TA and SSC:TA

The levels of SSC were significantly lowered in ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum treated with BC and NC, irrespective of formulations, as compared to control after 25 d cold storage (Table 6.6). In ‘Diamond Bright’ nectarine, the lowest SSC percent was observed in the fruit treated with BC fumigation (9.8 %) and NC fumigation (9.9 %). In ‘Tegan Blue’ plum, the lowest SSC percent (13.3 %) was found in fruit treated with BC aqueous solution with ethanol and Tween® 20. However, the level of SSC in ‘Princess Time’ peach was not significantly affected by any of the BC and NC treatments. The peach fruit treated with aqueous solutions with ethanol alone, and with Tween® 20 alone, exhibited the significantly least SSC percentage after 25 d cold storage.

The percentage of TA in ‘Diamond Bright’ nectarine fumigated with BC and NC were higher (1.3 and 1.4 fold, respectively) than the control fruit (Table 6.6). The nectarine fruit treated with aqueous solutions of NC with ethanol alone and all BC aqueous solutions showed a significantly higher percentage of TA (up to 1.2 fold) than control. However, the levels of TA in these fruits were not significantly different from the fruit treated with ethanol alone, Tween® 20 alone and, ethanol and Tween® 20 alone aqueous solutions. There was no significant difference in the levels of TA between the treated and untreated fruits in ‘Princess Time’ peach and ‘Tegan Blue’ plum (Table 6.6).

The nectarine fruit fumigated with NC, the peach fruit treated with BC aqueous solution with ethanol and Tween® 20, and the plum fruit treated with NC aqueous solution with ethanol and Tween® 20 exhibited the significantly lowest ratio of SSC and TA as compared to their respective controls (Table 6.6).

Table 6. 6 SSC (%), TA (%) and SSC/TA ratio of 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum affected by different formulations of ethylene antagonists BC and NC after 25 d cold storage.

Treatments	**SSC (%)**			**TA (%)**			**SSC/TA**		
	Princess Time	**Diamond Bright**	**Tegan Blue**	**Princess Time**	**Diamond Bright**	**Tegan Blue**	**Princess Time**	**Diamond Bright**	**Tegan Blue**
Control	12.3±0.0bcd	11.3±0.0f	15.2±0.0f	1.03±0.0	0.8±0.05a	1.7±0.02	12.0±0.3ab	14.6±0.3e	9.2±0.1bc
Ethanol(Eth.)	11.3±0.0a	10.4±0.1cd	15.1±0.0f	1.03±0.0	0.9±0.03cd	1.5±0.03	11.0±0.2a	11.1±0.4b	9.8±0.2c
Tween® 20(T-20)	11.5±0.0a	10.8±0.1e	14.6±0.1cde	1.01±0.0	0.9±0.03cd	1.5±0.04	11.5±0.1ab	11.6±0.4bc	9.8±0.2c
Eth. +T-20	12.2±0.0bcd	11.3±0.1f	14.9±0.1ef	1.09±0.1	0.9±0.05bc	1.6±0.10	11.2±0.5a	12.8±0.7cd	9.2±0.6bc
BC + Eth.	12.1±0.1bc	10.9±0.1e	14.2±0.1bc	1.03±0.0	0.9±0.03cd	1.7±0.00	11.8±0.3ab	11.7±0.4bc	8.5±0.02ab
BC + T-20	12.3±0.0cd	10.2±0.2bc	14.4±0.0bcd	0.98±0.0	0.9±0.05cd	1.6±0.02	12.6±0.2abc	11.1±0.8b	9.2±0.1bc
BC+ Eth. + T-20	12.4±0.1cd	10.2±0.2bc	13.3±0.2a	0.89±0.0	0.9±0.05c	1.6±0.04	13.9±0.2c	11.5±0.7bc	8.2±0.3ab
BC-Fumigation	12.3±0.1bcd	9.8±0.1a	14.1±0.2b	0.96±0.0	1.0±0.04cde	1.7±0.00	12.83±0.3bc	10.3±0.4ab	8.4±0.1ab
NC + Eth.	12.0±0.1b	10.6±0.0de	14.6±0.0cde	1.03±0.1	1.0±0.03de	1.7±0.02	11.9±0.8ab	10.6±0.9ab	8.8±0.1abc
NC + T-20	12.1±0.1bc	10.2±0.0bc	14.1±0.1b	1.01±0.0	0.7±0.03a	1.7±0.02	12.1±0.5ab	13.9±0.6de	8.5±0.1ab
NC+ Eth. + T-20	12.4±0.1d	10.8±0.1e	14.1±0.3bc	1.01±0.0	0.8±0.00ab	1.8±0.19	12.3±0.1ab	13.4±0.1de	7.9±0.6a
NC-Fumigation	12.2±0.1bcd	9.9±0.1ab	14.7±0.0de	1.03±0.0	1.1±0.02e	1.7±0.05	11.9±0.3ab	9.4±0.2a	8.9±0.3abc
LSD ($P \leq 0.05$)	0.231**	0.310 **	0.402 **	ns	0.088 **	ns	1.3**	1.3 **	1.1*

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

6.3.5 Individual sugars

Among the identified individual sugars, sucrose was observed to be the predominant sugar in 'Princess Time' peach and 'Diamond Bright' nectarine, while fructose was the predominant sugar in 'Tegan Blue' plum after 25 d cold storage (Table 6.7 and 6.8). The levels of glucose in the peach and nectarine fruits treated with different formulations of BC and NC were not significantly different to the control (Table 6.7), except the peach fruit treated with NC aqueous solutions with ethanol alone, and with ethanol plus Tween® 20. The glucose concentrations in the plum treated with BC and NC, regardless of formulations, were significantly higher up to 1.4 fold as compared to untreated fruit. The concentrations of fructose, sucrose and sorbitol were not significantly different between the treated and untreated peach fruits (Table 6.7 and 6.8). The peach fruit treated with the aqueous solution of ethanol plus Tween® 20 showed the highest fructose (13.12 g kg^{-1}), sucrose (113.2 g kg^{-1}) and sorbitol (3.1 g kg^{-1}) concentrations after 25 d cold storage. The significantly highest level of fructose (19.15 g kg^{-1}) was recorded in the nectarine fruit treated with Tween® 20 alone aqueous solution (Table 6.7). Similarly, the fructose levels of the plum fruit treated with NC irrespective of formulations, BC fumigation and BC aqueous solution with ethanol plus Tween® 20 were significantly higher than control after 25 d cold storage (Table 6.7). The sucrose levels of nectarine fruits treated with different formulations of BC and NC, except NC aqueous solution with ethanol alone, were maintained the same as in control fruit. The nectarine fruit treated with NC aqueous solution with ethanol alone exhibited a 1.2 fold higher sucrose level than the control (Table 6.8). The plum fruit treated with NC formulations, except NC aqueous solution with ethanol and Tween® 20, also retained 1.2 fold higher sucrose concentration as compared to control. The sorbitol concentrations of nectarine fruit treated with different formulations of BC and NC, except BC fumigation and NC aqueous solution with ethanol, were not significantly different from the control (Table 6.8). The nectarine fumigated with BC showed the lowest sorbitol content (2.21 g kg^{-1}), while the fruit treated with NC aqueous solution with ethanol resulted in the highest sorbitol content (2.98 g kg^{-1}). Similarly, the sorbitol concentrations of the plum fruit treated with NC irrespective to formulations, and BC fumigation were significantly higher up to 1.5 fold as compared to control after 25 d cold storage (Table 6.8).

Table 6. 7 Glucose and fructose contents of ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum affected by different formulations of ethylene antagonists BC and NC after 25 d cold storage.

Treatments	**Glucose (g kg^{-1})**			**Fructose (g kg^{-1})**		
	Princess Time	**Diamond Bright**	**Tegan Blue**	**Princess Time**	**Diamond Bright**	**Tegan Blue**
Control	6.2±0.03bcde	11.35±0.2a	34.5±1.1a	11.38±0.1	16.05±0.2a	52.1±2.9ab
Ethanol(Eth.)	6.8±0.11cde	11.40±0.2a	35.7±0.4a	11.69±0.1	16.88±0.4ab	47.2±1.2a
Tween® 20(T-20)	6.1±0.12bc	13.52±0.9c	36.7±0.9a	11.30±0.3	19.15±0.8c	52.9±1.4abc
Eth. +T-20	6.9±0.26de	13.09±0.3bc	41.3±1.4b	13.12±0.9	18.22±0.5bc	60.3±2.4cde
BC + Eth.	6.4±0.17bcde	11.69±0.4ab	42.1±1.6bc	12.15±0.7	16.99±0.4ab	55.6±2.1bcd
BC + T-20	7.0±0.12e	12.34±0.4abc	41.6±0.8bc	12.07±0.3	17.89±0.4bc	58.0±1.3bcde
BC+ Eth. + T-20	5.7±0.26ab	11.84±0.1ab	45.9±1.0cd	10.98±0.7	18.29±0.2bc	61.8±1.2def
BC-Fumigation	6.2±0.34bcd	11.77±0.4ab	43.9±1.2bc	11.59±0.8	17.21±0.4ab	61.5±1.4def
NC + Eth.	5.0±0.22a	11.04±0.1a	44.6±2.2bcd	10.41±0.6	16.93±0.2ab	65.3±3.8ef
NC + T-20	6.2±0.18bcde	12.34±0.1abc	48.7±1.1d	11.25±0.4	18.26±0.1bc	69.5±1.9f
NC+ Eth. + T-20	5.2±0.24a	11.36±0.2a	44.2±1.0bc	11.25±0.7	17.32±0.2ab	62.6±1.6def
NC-Fumigation	6.2±0.07bcd	11.91±0.4ab	45.7±1.3bcd	11.47±0.1	18.09±0.3bc	65.9±1.4ef
LSD ($P \leq 0.05$)	0.702**	0.042 *	3.989 **	ns	1.414 **	7.316 **

Means in the same column followed by the different letters are significantly different by Duncan’s multiple range test at $P \leq 0.05$. The letterings for ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 6. 8 Sucrose and sorbitol contents of ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum affected by different formulations of ethylene antagonists BC and NC after 25 d cold storage.

Treatments	**Sucrose (g kg^{-1})**			**Sorbitol (g kg^{-1})**		
	Princess Time	**Diamond Bright**	**Tegan Blue**	**Princess Time**	**Diamond Bright**	**Tegan Blue**
Control	99.5±3.3abc	78.33±1.3bcde	48.3±1.6b	2.55±0.01cd	2.54±0.03cd	37.8±2.4ab
Ethanol(Eth.)	82.2±2.9a	71.87±1.8abc	36.9±1.2a	2.23±0.05ab	2.44±0.06bcd	34.0±0.3a
Tween® 20(T-20)	103.3±4.8bc	65.57±3.3a	47.8±0.6b	2.13±0.05a	2.33±0.05ab	38.1±0.5ab
Eth. +T-20	113.2±6.2c	77.85±2.4bcde	57.0±0.8cd	3.01±0.04e	2.60±0.09d	45.5±1.4c
BC + Eth.	106.5±4.3bc	70.48±1.5ab	40.0±0.6a	2.69±0.05d	2.51±0.03bcd	41.1±0.8bc
BC + T-20	80.9±3.0a	83.49±3.2ef	47.8±0.3b	2.38±0.08abc	2.48±0.04bcd	39.8±0.4b
BC+ Eth. + T-20	106.6±3.2bc	81.38±2.2de	40.8±1.3a	2.44±0.05bcd	2.55±0.02cd	38.5±0.5ab
BC-Fumigation	100.2±7.7abc	75.84±1.8bcde	50.9±1.4bc	2.62±0.14cd	2.21±0.03a	44.4±0.1c
NC + Eth.	104.8±6.5bc	90.70±0.6f	57.8±3.6d	2.39±0.05abc	2.98±0.04e	50.5±2.1d
NC + T-20	91.1±2.4ab	73.74±1.7abcd	55.4±2.6cd	2.42±0.04bcd	2.38±0.03abc	54.7±1.6d
NC+ Eth. + T-20	107.1±8.4bc	79.63±0.8cde	49.1±0.6b	2.54±0.10cd	2.55±0.02cd	45.5±0.2c
NC-Fumigation	109.3±1.7bc	77.01±2.7bcde	55.9±2.6cd	2.49±0.01bcd	2.47±0.04bcd	50.7±1.0d
LSD ($P \leq 0.05$)	18.124 *	7.832 **	5.893 **	0.242 **	0.161 **	4.398 **

Means in the same column followed by the different letters are significantly different by Duncan’s multiple range test at $P \leq 0.05$. The letterings for ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

6.3.6 Individual organic acids

The malic acid was observed to be the major organic acid among the identified organic acids in all 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum after 25 d cold storage (Table 6.9 and 6.10). The concentration of individual organic acids in 'Diamond Bright' nectarine were comparatively lower than 'Princess Time' peach and 'Tegan Blue' plum. The level of malic acid in nectarine fruit treated with NC aqueous solution with ethanol alone was observed to be significantly the highest (4.01 g kg^{-1}), followed by the fruit fumigated with BC (3.71 g kg^{-1}) (Table 6.10). The peach fruit treated with BC and NC formulations, except BC aqueous solution with Tween® 20, retained significantly higher malic acid contents (up to 1.2 fold) as compared to control. Similarly, malic acid concentrations of the plum treated with NC formulations, except NC aqueous solution with ethanol plus Tween® 20, were maintained substantially higher (1.2 fold each) as compared to control. The levels of succinic acid in the nectarine fruit treated with BC and NC formulations, except BC aqueous solution with ethanol plus Tween® 20 and NC fumigation, were not significantly different to control (Table 6.9). Succinic acid in the nectarine fruit treated with BC aqueous solution with ethanol plus Tween® 20 and NC fumigation were significantly lower (1.1 fold each) as compared to control. Similarly, the plum fruit treated with BC and NC, irrespective of the formulations, showed significantly higher levels of succinic acid (up to 1.3 fold) as compared to the untreated plum. Effect of BC and NC formulations on fumaric and citric acids were varied in peach, nectarine and plum after 25 d cold storage (Table 6.10). BC fumigation maintained the significantly higher concentration of fumaric acid in nectarine, whilst it retained significantly lower fumaric acid content in peach and plum. However, NC fumigation and BC aqueous solution with ethanol treatments maintained significantly lower concentrations of fumaric acid as compared to control in all cultivars. Similar to fumaric acid, citric acid also responded differently to BC and NC formulations depending on the cultivar (Table 6.10).

Table 6. 9 Malic and succinic acid contents of ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum affected by different formulations of ethylene antagonists BC and NC after 25 d cold storage.

Treatments	**Malic acid (g kg^{-1})**			**Succinic acid (g kg^{-1})**		
	Princess Time	**Diamond Bright**	**Tegan Blue**	**Princess Time**	**Diamond Bright**	**Tegan Blue**
Control	13.01±0.5ab	3.47±0.05cd	22.13±0.4bc	2.65±0.1bc	2.05±0.05c	3.37±0.1a
Ethanol (Eth.)	12.51±0.1a	3.28±0.03bc	18.47±0.7a	2.92±0.2cd	1.94±0.05bc	3.37±0.1a
Tween® 20(T-20)	13.50±0.4bc	3.23±0.06b	22.29±0.2bc	2.89±0.1c	2.37±0.03d	3.85±0.1b
Eth. +T-20	16.38±0.2d	3.44±0.04cd	23.76±0.6bcd	2.45±0.1ab	1.98±0.04bc	3.84±0.1b
BC + Eth.	15.75±0.1d	3.49±0.05d	21.53±0.5b	2.32±0.1a	1.89±0.07ab	3.77±0.1b
BC + T-20	12.49±0.6a	3.35±0.04bcd	23.31±0.2bcd	2.94±0.1cd	2.07±0.05c	3.96±0.04bc
BC+ Eth. + T-20	14.05±0.6c	2.94±0.05a	23.66±0.2bcd	3.42±0.2e	1.80±0.04a	4.00±0.03bc
BC-Fumigation	15.61±0.1d	3.71±0.07e	23.56±0.4bcd	2.49±0.1ab	1.98±0.09bc	3.83±0.04b
NC + Eth.	15.41±0.1d	4.01±0.03f	25.33±1.3d	2.71±0.1bc	2.00±0.03bc	4.13±0.2bc
NC + T-20	14.31±0.2c	3.20±0.01b	25.42±0.6d	3.20±0.1de	2.09±0.04c	4.30±0.1c
NC+ Eth. + T-20	15.52±0.2d	3.32±0.04bcd	24.19±0.6cd	2.82±0.1c	1.99±0.04bc	4.08±0.1bc
NC-Fumigation	15.85±0.3d	3.37±0.11bcd	25.32±0.8d	2.51±0.1ab	1.90±0.09ab	4.29±0.1c
LSD ($P \leq 0.05$)	0.881 **	0.178 **	2.24 **	0.273**	0.132 **	0.384 **

Means in the same column followed by the different letters are significantly different by Duncan’s multiple range test at $P \leq 0.05$. The letterings for ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 6. 10 Fumaric and Citric acid contents of 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum affected by different formulations of ethylene antagonists BC and NC after 25 d cold storage.

Treatments	**Fumaric acid (g kg^{-1})**			**Citric acid (g kg^{-1})**		
	Princess Time	**Diamond Bright**	**Tegan Blue**	**Princess Time**	**Diamond Bright**	**Tegan Blue**
Control	0.225±0.0cd	0.080±0.0bc	0.275±0.0fg	4.08±0.1bcd	3.68±0.1c	0.389±0.01cde
Ethanol (Eth.)	0.247±0.0e	0.080±0.0bc	0.265±0.0de	3.84±0.3bc	3.52±0.1abc	0.347±0.01ab
Tween® 20(T-20)	0.248±0.0e	0.090±0.01d	0.290±0.0h	2.95±0.1a	4.47±0.1d	0.364±0.01abc
Eth. +T-20	0.217±0.0abc	0.085±0.0cd	0.283±0.0gh	4.01±0.1bc	3.72±0.1c	0.371±0.00abcd
BC + Eth.	0.213±0.0ab	0.061±0.0a	0.234±0.0a	4.31±0.3cde	3.61±0.1bc	0.382±0.0bcde
BC + T-20	0.232±0.0d	0.082±0.0bcd	0.257±0.0bc	4.60±0.2de	3.29±0.1ab	0.402±0.0de
BC+ Eth. + T-20	0.254±0.0e	0.065±0.0a	0.261±0.0cd	2.64±0.1a	3.52±0.04abc	0.352±0.02ab
BC-Fumigation	0.209±0.0a	0.089±0.0d	0.252±0.0b	3.65±0.1b	3.30±0.1ab	0.343±0.01a
NC + Eth.	0.225±0.0cd	0.075±0.0b	0.279±0.0g	2.98±0.1a	3.64±0.1bc	0.396±0.02cde
NC + T-20	0.216±0.0abc	0.084±0.0cd	0.270±0.0ef	4.81±0.2e	3.79±0.2c	0.409±0.01e
NC+ Eth. + T-20	0.224±0.0bcd	0.087±0.0cd	0.268±0.0def	3.01±0.2a	3.31±0.03ab	0.389±0.01cde
NC-Fumigation	0.211±0.0a	0.062±0.0a	0.232±0.0a	3.54±0.1b	3.25±0.1a	0.390±0.01cde
LSD ($P \leq 0.05$)	0.011**	0.008 **	0.008 **	0.501 **	0.310 **	0.032 *

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

6.3.7 Total phenols, ascorbic acid and total antioxidant capacity

Total phenolic contents of ‘Princess Time’ peach and ‘Diamond Bright’ nectarine fruit treated with different formulations of BC and NC were not significantly different to control fruit after 25 d cold storage (Table 6.11). The level of total phenols in ’Tegan Blue’ plum treated with NC irrespective of formulations, BC fumigation and BC aqueous solution with ethanol plus Tween® 20 were significantly lower up to 1.5 fold as compared to control fruit. The concentration of ascorbic acid in the nectarine fruit treated with different formulations of BC and NC were not significantly different to control (Table 6.11). Similarly, ascorbic acid of peach was not significantly affected by BC and NC formulations, except NC fumigation which maintained significantly lower ascorbic acid content (4.73 g kg^{-1}) compared to control. The plum treated with NC aqueous solution with ethanol plus Tween® 20 showed the significantly highest ascorbic content (17.9 g kg^{-1}), whilst the fruit treated with BC aqueous solution with Tween® 20 alone maintained the lowest ascorbic acid (14.8 g kg^{-1}). The different formulations of BC and NC, except BC fumigation, did not show any significant changes intotal antioxidant capacity of the peach, nectarine and plum after 25 d cold storage (Table 6.11). Total antioxidant capacity of the nectarine fruit fumigated with BC was significantly 1.2 fold higher as compared to control.

Table 6. 11 Total phenols, ascorbic acid and total antioxidant capacity of 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum affected by different formulations of ethylene antagonists BC and NC after 25 d cold storage.

Treatments	**Total phenols (g GAE kg^{-1})**			**Ascorbic acid (g kg^{-1})**			**Total Antioxidants capacity (mmol TEAC kg^{-1})**		
	Princess Time	**Diamond Bright**	**Tegan Blue**	**Princess Time**	**Diamond Bright**	**Tegan Blue**	**Princess Time**	**Diamond Bright**	**Tegan Blue**
Control	19.09±0.5	10.85±1.6	97.7±0.9cd	5.90±0.2b	9.91±0.18	16.3±0.5bcd	63.31±1.3	40.21±0.63bc	181.1±3.8
Ethanol(Eth.)	17.09±2.5	15.22±0.9	87.2±1.9bc	7.51±0.4d	10.23±0.56	16.1±0.3bcd	66.51±2.4	42.57±0.35c	189.8±12.6
Tween® 20(T-20)	16.09±0.6	11.85±2.4	101.1±5.3d	6.27±0.3bc	9.86±0.40	15.9±0.3abcd	65.09±1.6	35.57±0.80a	194.4±5.8
Eth. +T-20	14.6±2.8	13.47±2.0	99.8±4.5cd	5.99±0.2bc	10.21±0.29	15.2±0.3ab	71.67±1.4	40.64±0.81bc	188.4±9.2
BC + Eth.	15.84±1.1	15.47±4.8	99.7±1.4cd	6.83±0.1cd	9.95±0.45	15.2±0.5ab	56.38±0.2	38.30±0.70ab	171.6±7.1
BC + T-20	16.34±1.9	11.98±0.5	97.9±1.5cd	5.87±0.1b	8.90±0.25	14.8±0.3a	57.56±2.2	42.29±0.94c	171.8±5.9
BC+ Eth. + T-20	18.34±1.3	13.97±2.4	65.2±1.3a	6.18±0.1bc	9.09±0.17	16.5±0.2cd	61.53±4.45	39.25±1.06bc	183.7±4.8
BC-Fumigation	16.22±2.1	15.34±4.2	67.0±3.2a	5.94±0.1bc	9.58±0.20	16.5±0.3cd	61.35±2.4	47.04±2.71d	184.1±4.0
NC + Eth.	19.34±2.4	11.1±1.4	72.9±5.9a	5.94±0.3bc	9.02±0.13	15.8±0.3abc	58.80±1.5	41.21±0.35bc	178.1±4.1
NC + T-20	20.08±0.4	10.98±1.9	71.4±4.4a	5.62±0.3b	9.49±0.19	17.1±0.3de	64.54±2.7	40.90±0.84bc	184.3±1.0
NC+ Eth. + T-20	17.09±2.8	12.23±0.3	68.0±4.4a	5.87±0.3b	9.11±0.10	17.9±0.2e	63.38±3.5	40.44±0.70bc	188.6±6.1
NC-Fumigation	14.35±0.3	10.35±1.9	76.5±2.7ab	4.73±0.2a	9.72±0.30	16.2±0.2bcd	60.55±0.7	41.55±0.53bc	177.8±4.6
LSD ($P \leq 0.05$)	ns	ns	11.58 **	0.809 **	ns	1.04 **	ns	3.190 **	ns

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

6. 4 Discussion

The effect of different formulations of ethylene antagonists BC and NC on ethylene production and postharvest fruit quality were investigated in 'Princess Time' peach, 'Diamond Bright' nectarine and 'Tegan Blue' plum. The fumigation with BC or NC reduced ethylene production irrespective of cultivar and cold storage period, yet the effectiveness of BC and NC aqueous solutions were varied depending on the adjuvants, cultivar and cold storage period. In general, the fumigation treatments performed better than the aqueous solutions. Similarly, fumigation with gaseous cyclopropene for 24 h delayed chlorophyll degradation and fruit softening of banana, whilst aqueous solutions of cyclopropene salts showed their effectiveness only in the treated part of fruit Sisler et al. (2009). Being the aqueous solutions of cyclopropene salts not diffused from the treated area, the untreated part of banana ripened normally resulting in chlorophyll degradation and firmness reduction (Sisler et al., 2009). The diffusion or infiltration of BC and NC through the fruit surface as fumigants or as aqueous solutions is postulated in Fig. 6.9. When applied as fumigants, BC and NC freely diffuse through the stomata to reach the target cell where the ethylene receptors are located (Fig. 6.9 A). For BC and NC aqueous spray solutions without adjuvants, droplets are unlikely to diffuse through the stomata due to the large contact angle which creates poor adhesion with fruit surface. Stomata are the natural barrier which prevents the spontaneous infiltration of aqueous solutions (Fernandez and Eichert, 2009). In addition, according to Fernandez and Eichert (2009), the hydrophobicity of cuticle is another barrier for infiltration of aqueous solutions which, in our case, is the BC and NC droplets (Fig. 6.9 B). The presence of adjuvants reduces the surface tension by lowering the contact angle between the droplets and the cuticle which enhances the adhesion of BC and NC solutions. Moreover, the amphiphilic structure of adjuvants, with hydrophilic heads which can increase the water solubility of BC or NC and lipophilic tails which can disrupt the cuticle structure, may increase the permeability of cuticle (Patra et al., 2006 and Castro et al., 2014). Therefore, the BC/NC spray droplets could infiltrate to the target cell through the stomata or the cuticle upon storage (Fig. 6.9 C).

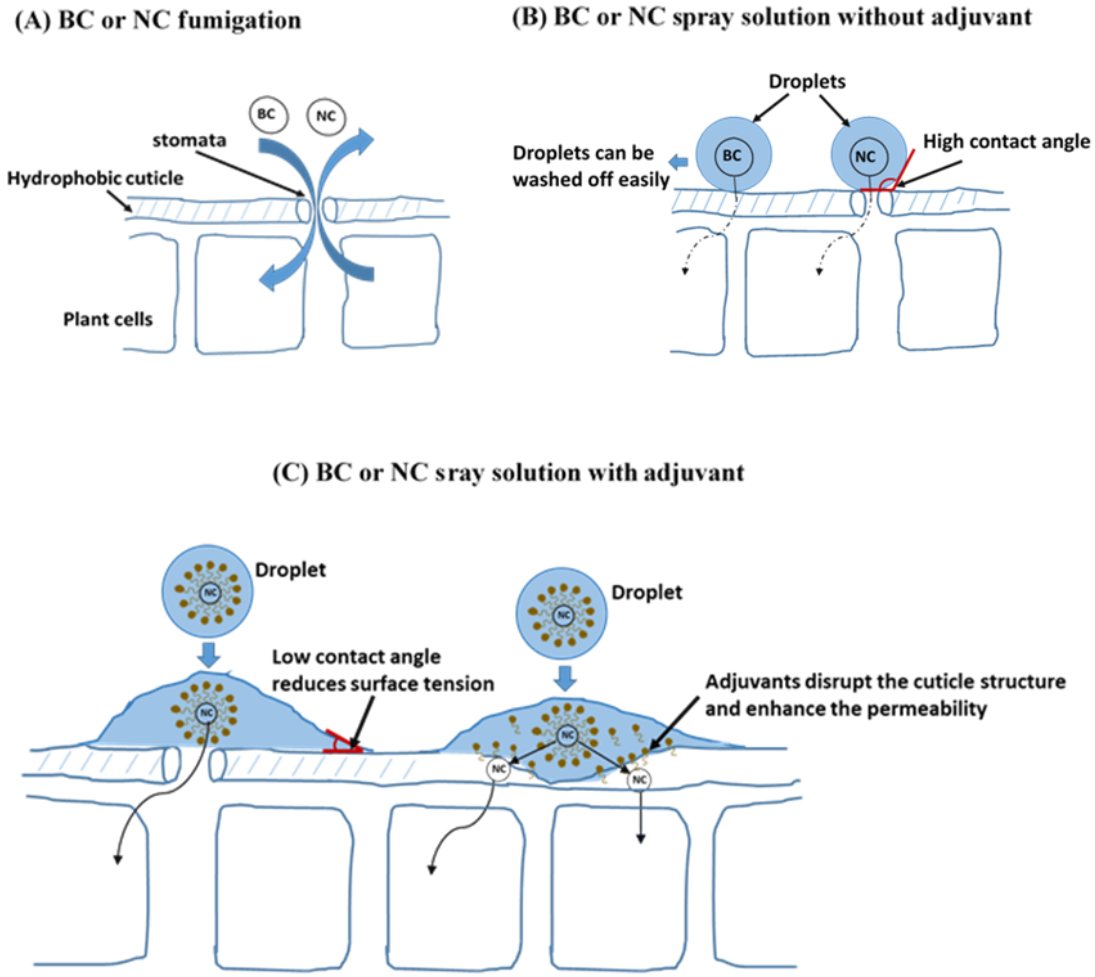

Fig 6. 9. The illustration of the diffusion, infiltration mechanisms of BC and NC formulations. (A) BC or NC fumigation, where gaseous BC/NC can easily diffuse through the fruit surface via stomata till the target cell. (B) BC or NC spray solution without adjuvant, where the infiltration of BC/NC droplets are limited due to the hydrophobicity of cuticle and the high contact angle between the droplets and the cuticle. (C) BC or NC spray solution with adjuvant, where the adjuvants enhance the solubility of BC/ NC and help to break the barrier properties of the fruit cuticle. In addition, they reduce the surface tension by lowering the contact angle of the spray droplets. Thus, the infiltration of aqueous solutions of BC/NC is increased through the cuticle or the stomata with the presence of adjuvants.

The suppression of ethylene production observed in BC and NC treatments are due to their proposed antagonistic effects to the ethylene receptors. As ethylene antagonists

can block the ethylene receptor binding sites, they suppress the physiological actions promoted by ethylene (Sisler and Serek, 1997 and Pirrung et al., 2008). A similar reduction of ethylene production following fumigation of ethylene antagonist has been reported in 'Maria Aurelia' nectarine treated with 1 µL L^{-1} 1-MCP for 24 h at 0°C (Ozkaya et al., 2016) and in 'Tegan Blue' plum treated with 1 or 2 µL L^{-1} 1-MCP for 24 h at 20±1°C (Khan and Singh, 2007). However, Fan et al. (2002) have reported that the effect of 1-MCP on ethylene production of 'Elberta' peach was varied depending on the harvest maturity and storage temperature. They observed that 1-MCP treatment (0.5 mL L^{-1} for 4 h at 20°C) was effective in reducing ethylene production of 'Elberta' peach when harvested 105 d after full bloom and stored at 20°C. In contrast to our result, application with different concentrations of 1-MCP (1-20 µL L^{-1}) at 20 °C for 5, 10 or 20 h was not effective in inhibition of ethylene production in 'Almog' and 'Oded' peaches (Liguori et al., 2004). Watkins (2006) also reported the effect of ethylene antagonist 1-MCP varied depending on species, cultivar, and concentration and exposure time of applied 1-MCP. On the other hand, the different responses of the present tested *Prunus* fruits to ethylene antagonists might be due to their variations in the number of ethylene receptor binding sites and the expression patterns of the ethylene receptor genes as previously postulated by Dal Cin et al. (2006) in 1-MCP treated 'Summer Rich' (SR) peach and 'Golden Delicious' (GD) apple. The ethylene biosynthesis of GD apple treated with 1 µL L^{-1} 1-MCP for 24 hr was totally suppressed up to 12 days. The ethylene climacteric peak of 1-MCP treated apple was also expressed only after 24 days of treatment whilst the control fruit expressed the peak after 12 days of 1-MCP treatment. However, the ethylene biosynthesis of SR peach did not respond to 1-MCP treatment. A similar trend was observed in the responses of ACC synthase, ACC oxidase and ethylene receptor genes, ETR1 and ERS1, to 1-MCP in this GD apple and SR peach.

The cultivar dependent variations in the effectiveness of BC and NC aqueous solutions may presumably be due to the differences in surface morphological structure, especially surface cuticle, of peach, nectarine and plum fruit (Fig. 6.10). It has been reported that there is a remarkable variation in the thickness of cuticle among the fruits but the significance of their functions has not been well understood (Martin and Rose,

2014). In ‘Tegan Blue’ plum, the ethanol in the aqueous solutions of BC and NC may have altered the structure of the surface cuticle right enough to diffuse BC and NC into the fruit tissues. In ‘Diamond Bright’ nectarine which apparently has thin fruit skin, the presence of 5% ethanol might have disrupted the surface cuticle diminishing the barrier properties of cuticle. In ‘Princess Time’ peach, the presence of trichome may have protected the fruit surface from the direct contact of BC and NC aqueous solutions. Fernandez et al. (2011) proposed that the trichome layer on the peach fruit surface serve as double hydrophobic protection. It may also influence the gaseous diffusion, the adhesion of water to the fruit surface. Further studies are warranted on the understanding of the functional properties fruit cuticles, as well as their potential role in postharvest treatments, especially in the applications of aqueous solutions.

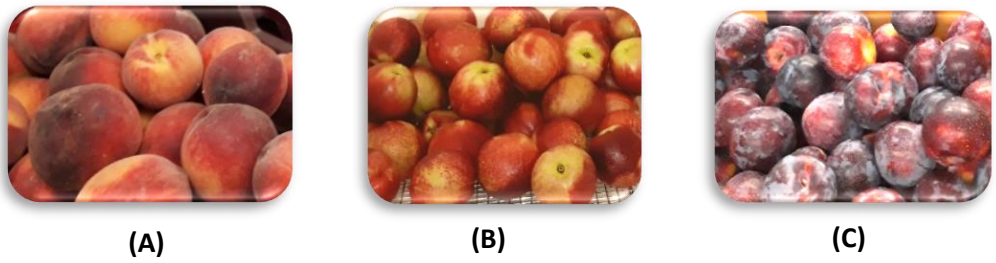

Figure 6. 10. The different surface morphology of *Prunus* fruits: (A) peaches with fuzzy surface, (B) nectarines with smooth surface and (C) plums with waxy surface.

The BC and NC did not show any effect on respiration rate of the tested peach, nectarine and plum. This is consistent with the previous findings of Liguori et al., (2004) in 1-MCP treated ‘Almog’ and ‘Oded’ peaches, and in ‘April Glow’ nectarine. Similarly, the ethylene antagonist 1-MCP had no significant effect on the respiration rate of ‘Black Amber’ plums stored for 45 d at 0°C (Ozkaya et al., 2010).

As the consequence of the reduction in ethylene production, the physiological weight loss of ‘Tegan Blue’ plum and ‘Diamond Bright’ nectarine fumigated with BC and NC were substantially reduced. Similarly, the fruit firmness of all the tested fruits were maintained higher. These results are in agreement with the findings of Martinez-Romero et al., (2003) where the weight loss and fruit firmness of the ‘Santa Rosa’ and ‘Golden Japan’ plums were significantly affected by the ethylene antagonist 1-MCP

at 1°C. Liguori et al., (2004) also reported that application with 5 µL L^{-1} of 1-MCP for 20 h inhibited the fruit softening of peaches and nectarines and maintained the fruit firmness up to 3 fold as compared to control fruit.

The soluble solids content of the nectarine and plum treated with BC and NC were maintained lower than the control although the titratable acidity and the SSC:TA were not different. The changes in the contents of SSC, TA and their ratio were determined by the levels of individual sugars and organic acid during fruit ripening (Valero and Serro, 2010a). The lower SSC contents may be the result of delayed fruit ripening induced by ethylene antagonists. Similar reduction in soluble solids content affected by ethylene antagonists were reported in 'Maria Aurelia' nectarine treated with 0.5 or 1 µL L^{-1} of 1-MCP for 24 h (Ozkaya et al., 2016) and in 'Songold' plum treated with 0.6 µL L^{-1} of 1-MCP for 24 h (Velardo-Micharet et al., 2017). However, the SSC level and titratable acidity content were higher in 'Yuhualu' peaches treated with 5 µL L^{-1} of 1-MCP for 24 h (Liu et al., 2015).

The sucrose content is the major sugar during the ripening of 'Princess Time' peach and 'Diamond Bright' nectarine as previously reported by Ramina et al. (2008) and Thakur and Singh (2012). However, in 'Tegan Blue' plum, fructose was found to be the predominant sugar which is consistent with the previous findings of Singh and Singh (2008) in Japanese plums. In the case of organic acids, malic acid was recorded as the major organic acid in all tested fruits, similar to the report of Singh et al. (2009). The accumulations of individual sugars and the breakdown of organic acids are the ethylene dependent biochemical changes during fruit ripening (Giovannoni, 2001). In the present study, the response of individual sugars and organic acids to the ethylene antagonists were varied depending on the species. The higher concentrations of individual sugars and higher malic acid concentrations in BC and NC treated plum might be the result of delayed fruit ripening induced by suppression in ethylene production. The suppression of ethylene with the fumigation of 1 µL L^{-1} of 1-MCP for 20 h maintained higher levels of the individual sugars and organic acids in 'Greensleeves' apples (Defilippi et al., 2004). Similar findings were reported in 'Stark Red Gold' nectarine Bregolie et al. (2005) and in 'Shushanggan' apricot Fan et al. (2018). In contrast, Bregoli et al. (2005) reported that the levels of sucrose and total

sugars were substantially lower in ‘Stark Red Gold’ nectarine fruit treated with 1 µL L^{-1} of 1-MCP for 12 h at 25°C.

In the present study, the levels of total phenols, ascorbic acid and total antioxidant capacity were relatively higher in plum compared to peach and nectarine. Similar variation in phenolic compounds was documented by Tomas-Barberan et al. (2001) in 25 different cultivars of peach, plum and nectarines; they reported that plum cultivars possess the comparative richer amount of phenolic compounds than the peach and nectarine cultivars. There was no significant effect of ethylene antagonists on the ascorbic acid and the total antioxidant capacity of the tested fruits. However, the total phenolic contents of ‘Tegan Blue’ plum treated with NC formulations and BC fumigation were maintained lower while the phenols of ‘Princess Time’ peach and ‘Diamond Bright’ nectarine did not respond to BC and NC treatments. Ethylene may presumably have a regulatory role in the metabolism of phenylalanine, the precursor of phenolic compounds, by enhancing the activity of phenylalanine ammonia lyase (PAL). Blankership and Unrath (1987) reported that the activity of PAL enzymes and the internal ethylene production were increased coincidently during the apple fruit maturation. The suppression of PAL activity with the application of nitric oxide, the ethylene biosynthesis inhibitor, was also noted in ‘Santa Rosa’ plum (Sharma and Sharma, 2015). Therefore, the reduction of total phenol contents in the present study may be attributed to the suppression of ethylene production by ethylene antagonists BC and NC. Consistent with the present finding, the phenolic contents of the ‘Booth 7’ avocado treated with aqueous 1-MCP were significantly suppressed although the antioxidant capacity was not remarkably different from the control fruit (Zhang et al., 2013). Similarly, the phenolic contents of ‘Yuhualu’ peach treated with 1-MCP exhibited lower phenolic contents compared to untreated fruit (Liu et al., 2015). However, the effect on ascorbic acid contents and the total antioxidant capacity from 1-MCP in these ‘Yuhualu’ peach were contradictory to the present results. The different responses of phenolic compounds, ascorbic acids and total antioxidant capacity to the ethylene antagonists in the tested peach, nectarine and plum fruits may be due to the differences in the composition of bioactive compounds depending on fruit type (Gil et al., 2002; Singh et al., 2010). Evaluation on 25 different cultivars of

peach, nectarine and plum revealed that plum contains more phenolic compounds, thus more antioxidant activity, compared to peaches and nectarines (Gil et al., 2002). In addition, harvest maturity, environmental conditions during postharvest handling and other pre-harvest factors also determine the composition and level of bioactive compounds in stone fruit (Cantin et al., 2009; Cosmulescu et al., 2015; Yalcin and Capar, 2017).

6. 5 Conclusion

In summary, the fumigation with ethylene antagonists BC and NC suppressed and delayed ethylene climacteric production, reduced weight loss and retained higher fruit firmness of all the tested *Prunus* species at 20±1°C (ambient temperature) or after 25 d storage at 1°C. Unlike fumigation treatments, the effectiveness of aqueous solutions of BC and NC on ethylene production, weight loss and fruit firmness were different depending on the fruit species. 'Tegan Blue' plum and 'Diamond Bright' nectarine responded more positively to the aqueous solutions of BC and NC than 'Princess Time' peach. The effect on other fruit quality parameters to the BC and NC fumigations and aqueous solutions were varied depending on the formulations and the fruit species. The results indicated the potential of BC and NC fumigations in controlling fruit ripening and maintaining postharvest fruit quality of the tested *Prunus* fruits. However, future research is required to optimise the effectiveness of aqueous solutions of BC and NC for each specific fruit species.

CHAPTER 7

Ethanol enhances the performance of 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) aqueous solutions in managing fruit ripening of nectarine and plum

Summary

The effect of different concentrations of ethanol to enhance the performance of aqueous solutions of ethylene antagonists 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) in controlling fruit ripening of nectarine and plum was investigated. Two separate experiments were conducted on 'Ruby Diamond' nectarine and 'Tegan Blue' plum. The fruits were fumigated with 1 µM of BC and NC for 18 h or sprayed with 2 µM of BC and NC aqueous solutions prepared with respective 2.5, 5.0 or 10.0 % ethanol at 20±1°C. The treated and untreated (control) fruit were then kept for 25 d at 1°C and 90±5% RH. The ethylene production and respiration rate were monitored daily for 8 d at 0 d or 25 d after cold storage. The changes in ripening related physiochemical attributes, i.e., weight loss, firmness, soluble solids content, titratable acidity, individual sugars and organic acids, ascorbic acid, total phenols and the total antioxidant capacity were evaluated only after 25 d cold storage. Overall, the effects of aqueous solutions of BC and NC on climacteric ethylene production, weight loss, firmness, soluble solids content, titratable acidity, individual sugars and organic acids were varied depending on the species and the concentrations of ethanol present. The presence of ethanol at a concentration of either 2.5 or 5% enhanced the effectiveness of aqueous solutions of BC and NC in reducing the climacteric ethylene production and in maintaining the fruit quality of 'Tegan Blue' plum. Unlike aqueous solutions, the fumigation treatments with BC and NC suppressed the climacteric ethylene production and maintained the fruit quality in both nectarine and plum. The levels of total phenols, ascorbic acids and the total antioxidant capacities of nectarine and plum fruits treated with BC and NC, regardless of the treatments, were preserved similar to the contents of control after the cold storage.

7.1 Introduction

Ripening is the final stage of fruit development followed by the quality deterioration mechanism called senescence, which ultimately leads to the death of cells (Giovannoni, 2001). A series of physiochemical changes such as degradation of chlorophyll, modification of cell wall structure and the development of flavour and aroma compounds through sugars and acids metabolism occurs during fruit ripening (Giovannoni, 2004 and 2008). Ethylene is responsible for most of the ripening-related quality deterioration processes (Burg and Burg, 1965), although not all the ripening-related processes are ethylene-dependent (Lelievre et al., 1997; Abdi et al., 1998). Ethylene is a gaseous compound which can be produced in all parts of the plant. In fruit, ethylene is produced starting from the fruit development stage and the production rate is dramatically increased during ripening, particularly in climacteric fruit type, triggering the subsequent senescence processes (Burg and Burg, 1965; Alexander and Grierson, 2002). As a consequence, the storage life is significantly reduced with the loss of aesthetic and nutritional quality values of the fruit (Valero et al., 2003). Nectarine and plum are climacteric fruits which exhibit a rapid fruit ripening with a limited potential storage life after harvest (Crisosto and Day, 2012). The storage life of nectarine and plum is about a week under ambient condition and it can be extended up to 3-5 weeks under cold storage depending on the cultivars (Mitchell et al., 1974; Khan and Singh, 2009).

The application of ethylene antagonists has been documented as one of the effective postharvest technologies to improve cold storage life. The beneficial effect of the ethylene antagonist, 1-methylcyclopropene (1-MCP) on ripening related physiology and biochemical changes were reported in ‘President’ plum (Valero et al., 2003), in ‘Stark Red Gold’ nectarine (Bregoli et al., 2005), in ‘Tegan Blue’ plum (Khan and Singh, 2009), in ‘Yuhualu’ peach (Liu et al., 2015) and in ‘Yulu’ and ‘Zajiao’ peaches (Yu et al., 2017).

It has also been proposed that the ethylene antagonist 1*H*-cyclopropabenzene (BC) and its derivative 1*H*-cyclopropa[*b*]naphthalene (NC) can delay fruit ripening process in some cultivars of plum and nectarine (Singh et al., 2018). According to Singh et al. (2018), BC and NC can be applied through fumigation, like 1-MCP, to control fruit ripening by blocking the action of ethylene at the receptor level. Being applied as

fumigants, the postharvest application of these compounds are limited to certain conditions where the air-tight facilities are available. In addition, the ethylene antagonists in the form of aqueous solutions are also required for the broader use of these compounds in the open field conditions to overcome the deleterious effects of ethylene.

Unlike 1-MCP, BC and NC are relatively more stable at room temperature which makes them easier to handle and opens an opportunity to apply as aqueous solutions. However, they have limited solubility in water like other hydrocarbons. Groves (1988) has reported that the aqueous solubility of hydrocarbons such as benzene can be increased in the presence of co-solvent ethanol. Grichko (2006) also demonstrated that ethanol is the best candidate for the preparation of water-soluble ethylene antagonists. Ethanol can interrupt the barrier properties of the fruit cuticle by solubilising the lipid layers of the fruit cuticles (Farag et al., 1992). The presence of ethanol in preparation of BC and NC aqueous solutions may promote the efficacy of BC and NC by increasing the delivery of these compounds across the fruit surface. Therefore, this experiment was designed to investigate the effect of different concentrations of ethanol on the performance of BC and NC in controlling the ripening process of nectarine and plum. It was hypothesised that the presence of ethanol may enhance the aqueous solutions of BC and NC as effective as the fumigation treatments in slowing down the ripening processes.

7.2 Materials and methods

7.2.1 Experimental conditions

The potential effect of ethanol on enhancing the effectiveness of aqueous solutions of 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) aqueous solutions in reducing ethylene production and maintaining fruit quality was investigated. Two separate experiments were conducted using ‘Ruby Diamond’ nectarine and ‘Tegan Blue’ plum. The information on the source of the fruit sample and the applied ethylene antagonists were mentioned in Chapter 3, section 3.3.1.

The first experiment was conducted in January 2018 at the Horticulture research lab, at Curtin University. 'Ruby Diamond' nectarine fruit were collected from the Eastwind orchard at commercial harvest stage (10.7±0.1 % TSS, 2.0±0.0 % TA and 40.5±1.5 N firmness). The fruit for the study (486) which were free from any physiological injuries and defects were selected and divided into nine groups. In our previous experiments, it was confirmed that the application of ethanol alone in aqueous solution has no significant effect on ethylene production. In this experiment, therefore, the selected fruits were sprayed with BC and NC aqueous solutions (2 µM) containing different concentrations of ethanol (2.5, 5 and 10 %, respectively) or fumigated with BC and NC (1 µM) for 18 h at 20±1°C. The untreated fruit were considered as control. The experiment was set up using the randomised completely blocked design with three replications. The BC and NC aqueous solutions with ethanol were prepared as described in Section 3.3. The treated and untreated fruit were stored for 25 d at 1 °C and 90±5 %RH after treating with the respective spray solutions. The physiological and fruit quality parameters were checked at the end of cold storage.

Similarly, the second experiment was carried out in February 2018 using 'Tegan Blue' plum (11.9±0.1 % TSS, 2.3±0.1 % TA and 39.5±1.7 N fruit firmness). The same experimental condition and the experimental procedure of experiment 1 were followed to conduct the second experiment.

7.2.2 Determination of physiological parameters

7.2.2.1 Ethylene production (nmol kg^{-1} h^{-1} and µmol kg^{-1} h^{-1})

Ethylene production of 'Ruby Diamond' nectarine was determined by using gas chromatography while that of 'Tegan Blue' plum was determined by a laser-based ethylene detector ETD-300 following the respective procedures described by Khan and Singh (2008) and Azzu (2016). The determination of ethylene production was described in detail in Chapter 3, section 3.5.1.

7.2.2.2 Respiration rate (mmol CO_2 kg^{-1} h^{-1})

The respiration rate was determined as the CO_2 production rate by using a gas analyser following the detailed procedure described by Khan and Singh (2008). Determination of respiration rate was described in Chapter 3, section 3.5.2.

7.2.2.3 Physiological weight loss (%) and fruit firmness (N)

Physiological weight loss was determined according to the formula described by Azzu (2016). The procedure for the calculation of physiological weight loss was described in Chapter 3, section 3.5.3.

Fruit firmness was determined following the procedure previously described by Khan and Singh (2008) as mentioned in Chapter 3, section 3.5.4.

7.2.3 Determination of quality parameters

7.2.3.1 SSC (%), TA (%) and SSC:TA

The levels of SSC, TA and SSC:TA ratio were determined by following the method described by Khan and Singh (2008). The detailed procedures for quantification of SSC (%), TA (%) and SSC:TA ratio were described in Chapter 3, section 3.6.1.

7.2.3.2 Individual sugars and organic acids (g kg^{-1})

The quantification of individual sugar and organic acid was done following the detailed procedure described by Usenik et al. (2008). The individual sugars i.e. glucose, fructose, sucrose and sorbitol, and the individual organic acids i.e. malic, citric, fumaric and succinic acids were identified using high-performance liquid chromatography (HPLC). The detailed procedures for sample preparation and quantification of individual sugars and organic acids were mentioned in Chapter 3, section 3.6.2.

7.2.3.3 Total phenols (g kg^{-1})

The quantification of total phenolic content was carried out following the procedure described by Cantin et al. (2009) with some modifications. The procedure for the extraction and the assessment of phenolic compound were mentioned in Chapter 3, section 3.6.3.

7.2.3.4 Ascorbic acid (g kg^{-1})

The ascorbic acid content was estimated following the procedure described by Vithana (2017). The procedure for the extraction and assessment of ascorbic acid were described in Chapter 3, section 3.6.4.

7.2.3.5 Total antioxidant capacity (mmol TEAC kg^{-1})

The total antioxidant capacity was determined by measuring the free radical scavenging capacity following the DPPH method as previously described by Vithana (2017) based on Brand-Williams et al. (1995). The preparation of the standard free radical solution, the extraction and quantification antioxidant capacity were detailed in Chapter 3, section 3.7.5.

7.2.4 Statistical analysis

The Genstat software (13th edition), Genstat release 13.1, VSN International Ltd., UK was used to analyse the collected data. The least significant differences (LSD) at ($P \leq 0.05$) was calculated to check the treatment effects. The data were presented as means ± standard errors of means and Duncan's multiple range test was used to compare the means of the treatments.

7.3 Results

7.3.1 Ethylene production

Ethylene production of 'Ruby Diamond' nectarine and 'Tegan Blue' plum were generally suppressed by BC and NC treatments, except aqueous solutions with 10% ethanol, after 0 d and 25 d storages at 1°C (Fig. 7.1 and 7.2). The ethylene climacteric peak concentration of 'Ruby Diamond' nectarine fruit fumigated with BC and NC were significantly reduced (two-fold each) as compared to control after 0 d at 1°C (Table 7.1). After 25 d at 1°C, the nectarine fruits fumigated with BC and NC showed a comparatively lower amount of ethylene climacteric peak concentration than control (Table 7.2). In 'Tegan Blue' plum, the fruit treated with BC and NC, except aqueous solutions with 10% ethanol and BCE 2.5%, expressed significantly lower amount of ethylene climacteric peak concentration as compared to control in both storage

conditions (Table 7.1 and 7.2). The plum fumigated with BC and NC exhibited lowest peak concentrations after 0 d at 1°C (1.3 nmol kg^{-1} h^{-1} each) and after 25 d at 1°C (2.1 and 1.7 nmol $kg^{-1}h^{-1}$, respectively). The onsets of ethylene climacteric peak were not significantly delayed by BC and NC treatments in 'Ruby Diamond' nectarine in both storage conditions and in 'Tegan Blue' plum after 0 d at 1°C. However, climacteric peak onsets of the plum fumigated with BC and NC were shown to be significantly delayed (2 and 3 d, respectively) as compared to control after 25 d storage at 1°C.

7.3.2 Respiration rate

The climacteric respiration peak rate of 'Ruby Diamond' nectarine and 'Tegan Blue' plum treated with different formulations of BC and NC were not significantly different from the untreated fruits in both storage condition (Table 7.3 and 7.4, Figure 7.4). Similarly, the onsets of respiration peak of the treated and untreated nectarine and plum were not significantly different from each other, except in the plum stored after 0 d at 1°C. The plum treated with BC and NC, irrespective of the formulations, delayed the onset of the respiration peak up to 2 d as compared to control after 0 d at 1°C (Table 7.3). The nectarine fruit treated with BC fumigation exhibited the lowest respiration rate throughout the ripening periods after 0 d at 1°C (Fig. 7.3).

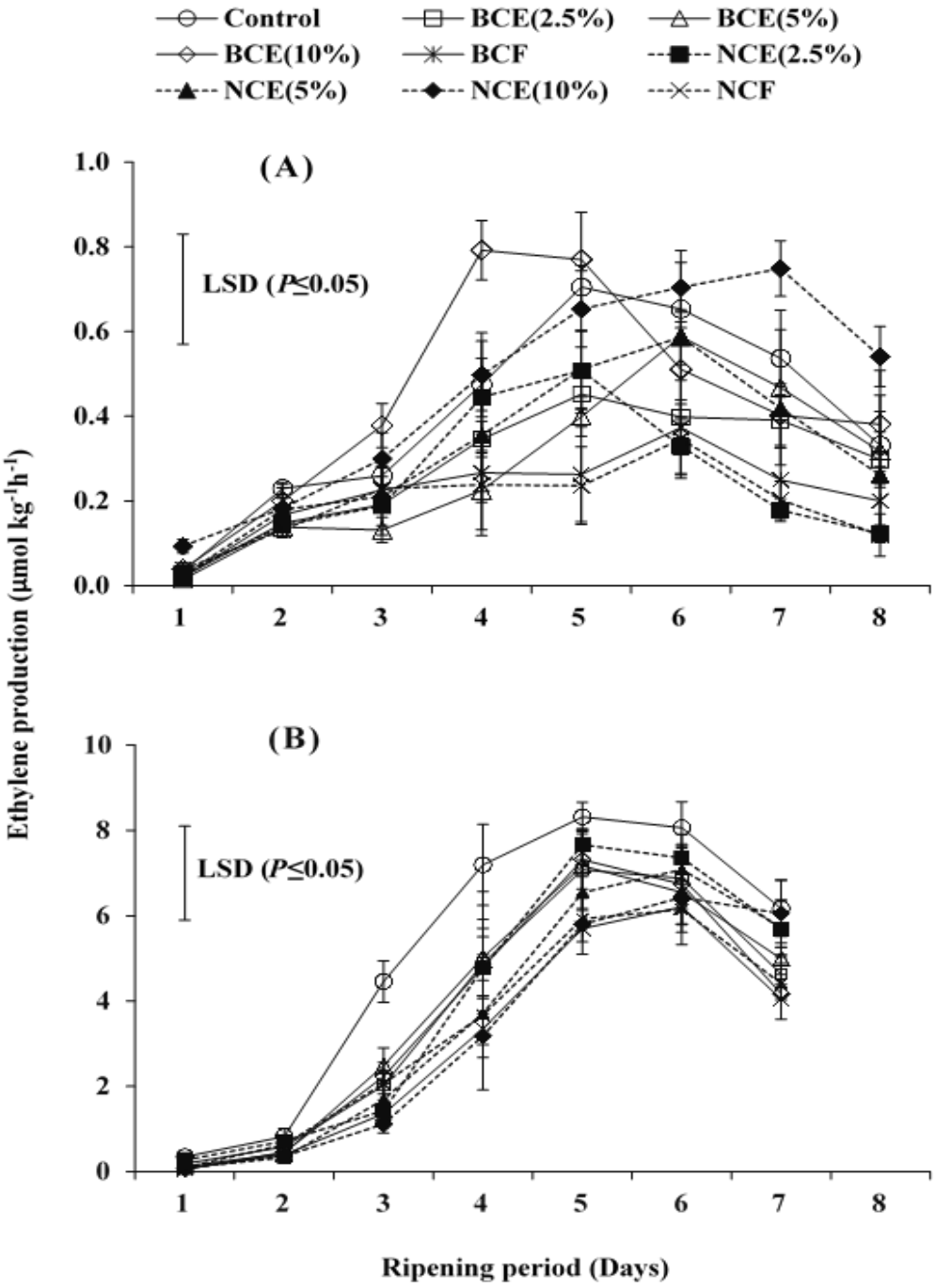

Fig. 7. 1 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on ethylene production of 'Ruby Diamond' nectarine (A) after 0 d and (B) after 25 d cold storage at 1°C. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for ethylene production of (A): treatments (tr) =0.09, days after storage (d) =0.08 and their interaction (tr x d) =ns, and (B): tr=0.77, d=0.68 and tr x d= ns.

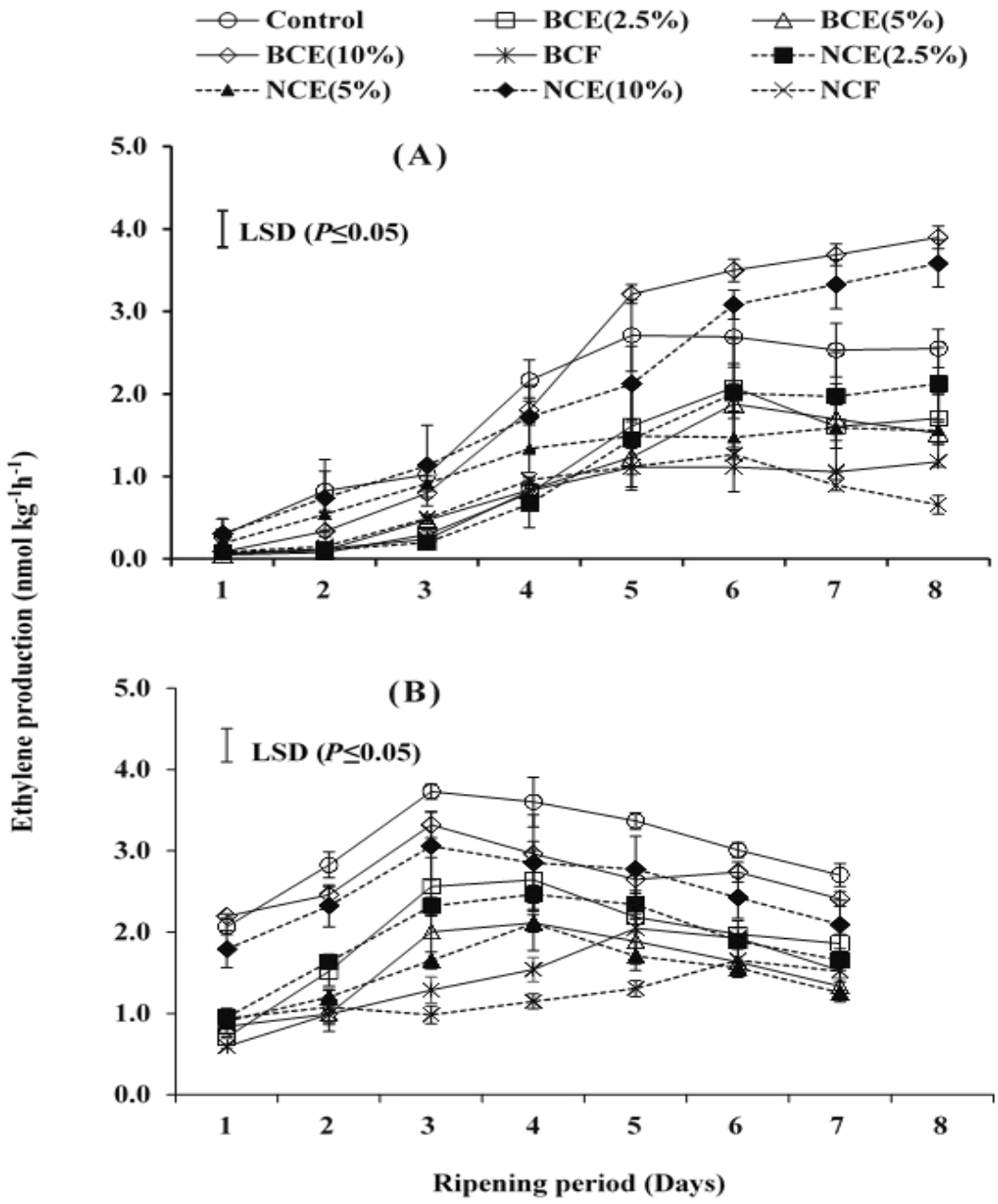

Fig. 7. 2 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on ethylene production of 'Tegan Blue' plum nectarine (A) after 0 d and (B) after 25 d cold storage at 1°C. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for ethylene production of (A): treatments (tr) =0.28, days after storage (d) =0.26 and their interaction (tr x d) =78, and (B): tr=0.25, d=0.22 and tr x d=ns.

Table 7. 1 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10 %), and fumigations on the ethylene climacteric peak onset and the ethylene climacteric peak concentration of 'Ruby Diamond' nectarine and 'Tegan Blue' plum after 0 d at 1°C.

Treatments	Ethylene climacteric peak onset		Ethylene climacteric peak concentration	
	Ruby Diamond (d)	Tegan Blue (d)	Ruby Diamond (μmol $kg^{-1}h^{-1}$)	Tegan Blue (nmol $kg^{-1}h^{-1}$)
Control	5.7±0.3	5.3±0.3	0.8±0.0bcd	2.9±0.3bc
BCE (2.5%)	5.7±0.5	6.0±0.0	0.5±0.0ab	2.1±0.0ab
BCE (5%)	6.0±0.0	6.3±0.3	0.6±0.1abc	2.0±0.3a
BCE (10%)	4.7±0.3	5.3±0.3	0.8±0.1cd	3.3±0.0c
BCF	6.0±0.0	6.0±0.5	0.4±0.1a	1.3±0.3a
NCE (2.5%)	5.3±0.3	5.7±0.3	0.5±0.1ab	1.8±0.5a
NCE (5%)	6.0±0.0	5.3±0.3	0.6±0.0abc	1.7±0.0a
NCE (10%)	6.0±0.5	5.7±0.3	0.9±0.0d	3.0±0.3c
NCF	6.0±0.0	5.7±0.3	0.4±0.1a	1.3±0.0a
LSD ($P \leq 0.05$)	ns	ns	0.29*	0.85**

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Ruby Diamond' and 'Tegan Blue' are independent from each other. **, * = significant at 1 % and 5 % level of LSD, ns=non-significance.

Table 7. 2 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10 %), and fumigations on the ethylene climacteric peak onset and the ethylene climacteric peak rate of 'Ruby Diamond' nectarine and 'Tegan Blue' plum after 25 d at 1°C.

Treatments	Ethylene climacteric peak onset		Ethylene climacteric peak concentration	
	Ruby Diamond (d)	Tegan Blue (d)	Ruby Diamond (μmol $kg^{-1}h^{-1}$)	Tegan Blue (nmol $kg^{-1}h^{-1}$)
Control	4.7±0.3	3.3±0.3ab	8.9±0.4	3.9±0.2d
BCE (2.5%)	5.3±0.3	4.0±0.0bc	7.3±0.8	2.6±0.4abc
BCE (5%)	5.0±0.0	4.3±0.3c	7.2±0.8	2.1±0.3ab
BCE (10%)	5.3±0.3	3.0±0.0a	7.5±0.3	3.3±0.3cd
BCF	6.0±0.0	5.3±0.3d	6.2±0.9	2.1±0.2ab
NCE (2.5%)	5.0±0.5	4.0±0.5bc	8.4±0.3	2.5±0.1abc
NCE (5%)	5.3±0.3	4.0±0.0bc	7.7±1.3	2.1±0.2ab
NCE (10%)	6.0±0.5	3.3±0.3ab	6.6±0.9	3.2±0.5cd
NCF	5.3±0.3	6.0±0.0d	6.4±0.8	1.7±0.2a
LSD ($P \leq 0.05$)	ns	0.83**	ns	1.0*

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Ruby Diamond' and 'Tegan Blue' are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

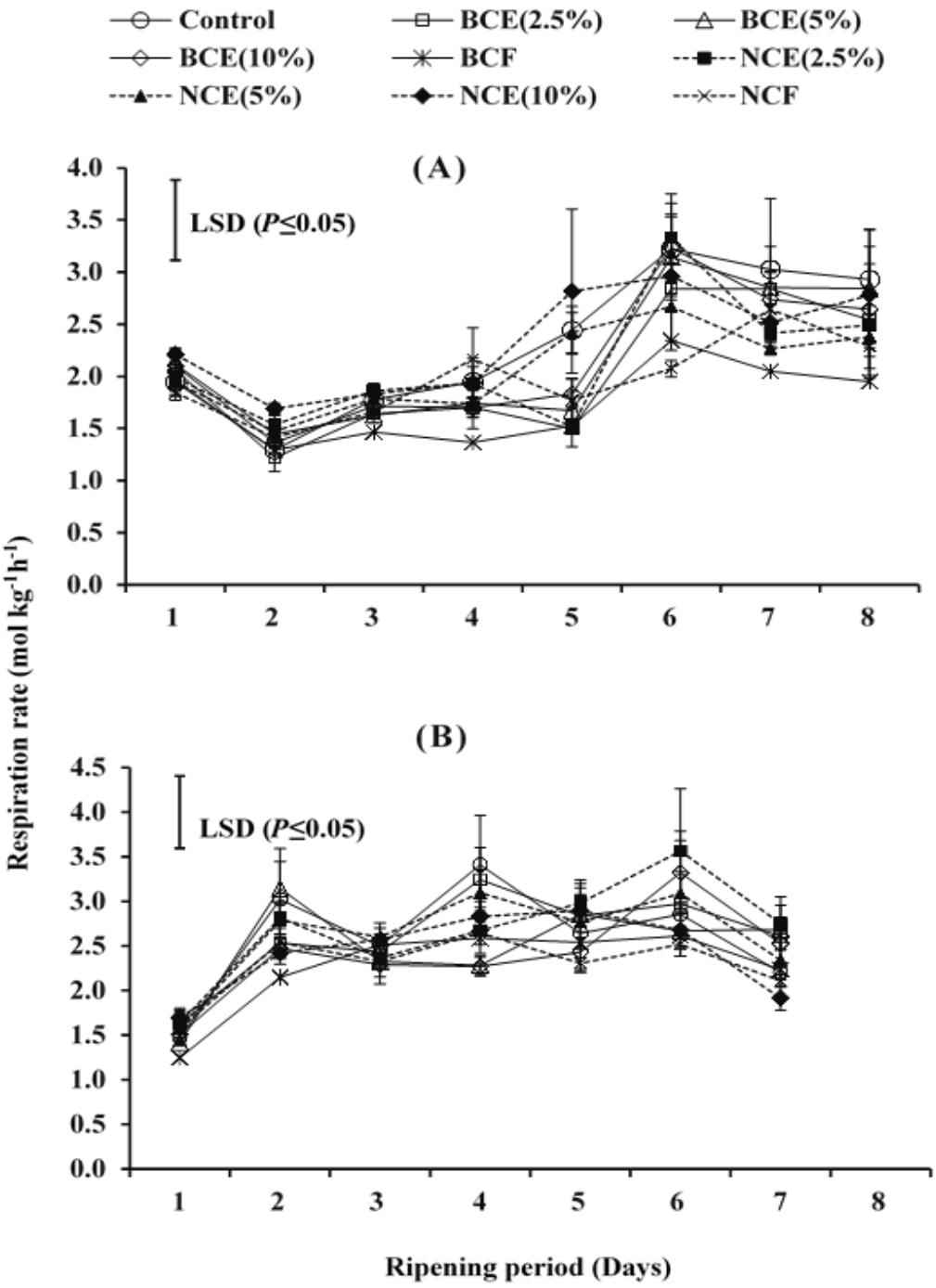

Fig. 7. 3 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on the respiration rate of 'Ruby Diamond' nectarine (A) after 0 d and (B) after 25 d cold storage at 1°C. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for respiration rate of (A): treatments (tr) =0.29, days after storage (d) =0.27 and their interaction (tr x d) =ns, and (B): tr=ns, d=0.29 and tr x d= 0.5.

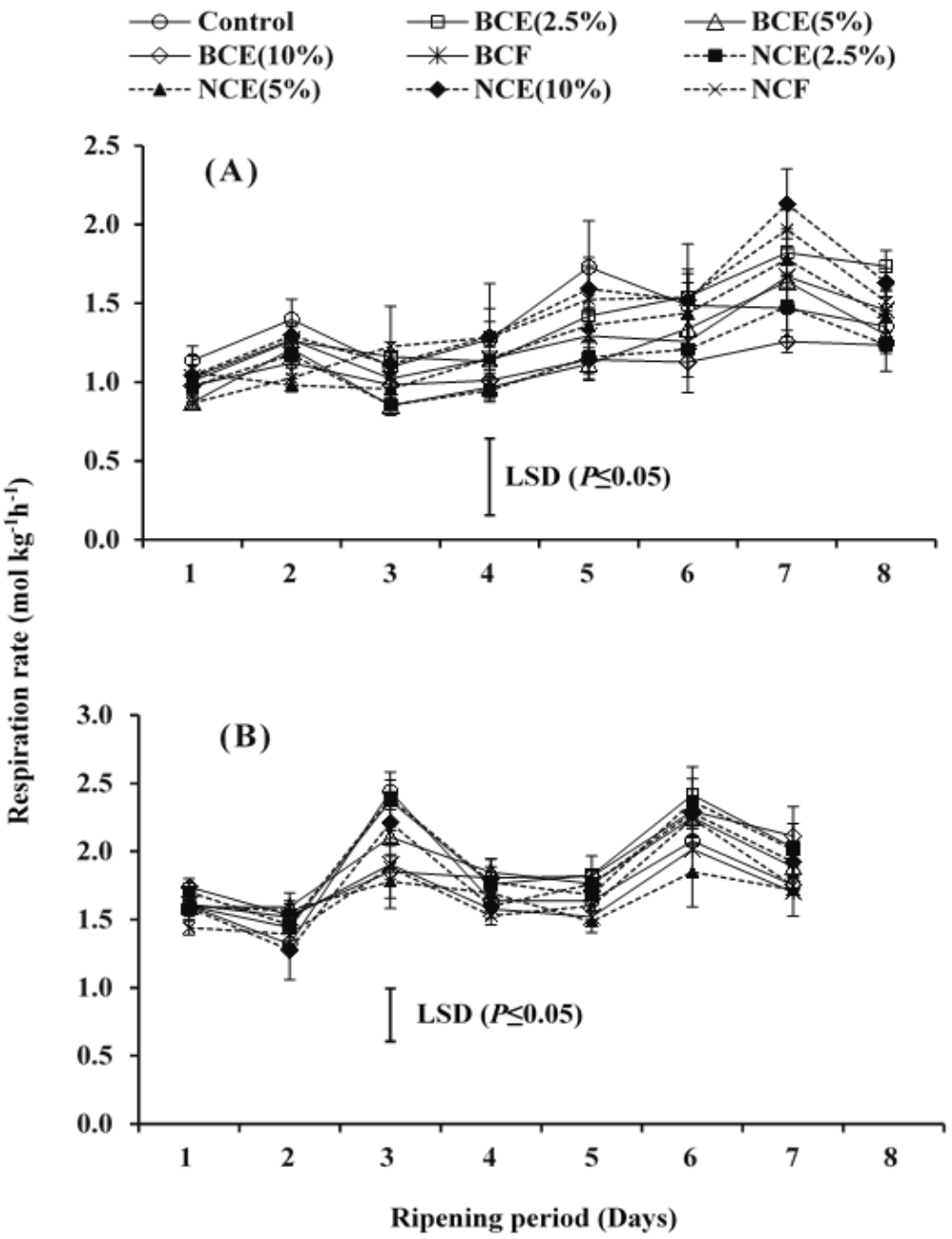

Fig. 7. 4 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on the respiration rate of 'Tegan Blue' plum nectarine (A) after 0 d and (B) after 25 d cold storage at 1°C. LSD bars represent treatments (tr) x days after storage (d). Vertical bars represent SE of means of three replicates and are not visible when the values are too small. LSD values for respiration rate of (A): treatments (tr) =0.17, days after storage (d) =0.16 and their interaction (tr x d) =ns, and (B): tr=0.15, d=0.13 and tr x d=ns.

Table 7. 3 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on the respiration climacteric peak onset and the respiration climacteric peak rate of 'Ruby Diamond' nectarine and 'Tegan Blue' plum after 0 d at 1°C.

Treatments	Respiration climacteric peak onset (d)		Respiration climacteric peak rate (mmol $kg^{-1}h^{-1}$)	
	Ruby Diamond	Tegan Blue	Ruby Diamond	Tegan Blue
Control	6.0±0.5	5.3±0.3a	3.4±0.5	1.8±0.3
BCE (2.5%)	6.3±0.3	7.7±0.3bd	2.9±0.5	1.9±0.0
BCE (5%)	6.3±0.3	7.0±0.0bcd	3.2±0.4	1.6±0.0
BCE (10%)	6.3±0.3	7.3±0.3bcd	3.3±0.2	1.3±0.1
BCF	6.0±0.0	7.3±0.3bcd	2.3±0.3	1.7±0.1
NCE (2.5%)	6.0±0.0	6.7±0.3b	3.3±0.4	1.5±0.2
NCE (5%)	6.0±0.0	6.7±0.3bc	2.7±0.2	1.8±0.4
NCE (10%)	6.0±0.5	7.0±0.0bcd	3.5±0.5	2.1±0.2
NCF	6.3±0.5	6.7±0.3bc	2.7±0.4	2.0±0.1
LSD ($P \leq 0.05$)	ns	0.88*	ns	ns

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Ruby Diamond' and 'Tegan Blue' are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 7. 4 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on the respiration climacteric peak onset and the respiration climacteric peak rate of 'Ruby Diamond' nectarine and 'Tegan Blue' plum after 25 d at 1°C.

Treatments	Respiration climacteric peak onset (d)		Respiration climacteric peak rate (mmol $kg^{-1}h^{-1}$)	
	Ruby Diamond	Tegan Blue	Ruby Diamond	Tegan Blue
Control	3.3±0.5	3.0±0.0	3.5±0.5	2.4±0.1
BCE (2.5%)	4.0±0.9	5.0±0.8	3.6±0.3	2.4±0.0
BCE (5%)	4.3±0.5	5.0±0.8	3.0±0.3	2.3±0.1
BCE (10%)	4.7±1.1	6.0±0.0	3.4±0.4	2.3±0.1
BCF	4.0±0.5	5.0±0.8	2.8±0.1	2.0±0.2
NCE (2.5%)	3.7±0.7	4.3±0.7	3.1±0.2	2.1±0.2
NCE (5%)	4.0±0.9	5.0±0.8	3.5±0.2	2.5±0.1
NCE (10%)	4.7±0.3	5.0±0.8	3.1±0.1	2.4±0.1
NCF	4.0±0.9	6.0±0.0	2.9±0.1	2.2±0.1
LSD ($P \leq 0.05$)	ns	ns	ns	ns

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Ruby Diamond' and 'Tegan Blue' are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

7.3.3 Physiological weight loss and firmness

The fumigation with ethylene antagonists reduced weight loss of ‘Ruby Diamond’ nectarine stored for 25 d at 1°C (Fig. 7.5 A). However, weight loss of ‘Tegan Blue’ plum did not respond to any of the BC and NC formulations (Fig. 7.5 B). Weight loss of ‘Ruby Diamond’ nectarine fumigated with BC and NC were significantly the lowest and 1.2 fold lower as compared to control fruit (Fig. 7.5 A). Likewise, the plum fumigated with BC and NC also exhibited comparatively lower weight loss than control after 25 d cold storage.

The ethylene antagonists, regardless of the formulations, maintained significantly higher fruit firmness in ‘Ruby Diamond’ nectarine (Fig. 7.6 A). The highest firmness retention (46 N) was observed in the nectarine fumigated with NC, followed by the fruit treated with NC aqueous solution with 5% ethanol (44.8 N) and the fruit fumigated with BC (44.6 N). In ‘Tegan Blue’ plum, only firmness of the fruit treated with the NC fumigation, NC aqueous solution with 5% ethanol and BC fumigation were significantly lower than the control after 25 d cold storage (Fig. 7.6 B). The plums fumigated with NC and BC showed significantly highest fruit firmness which were 1.6 and 1.5 fold, respectively higher than control.

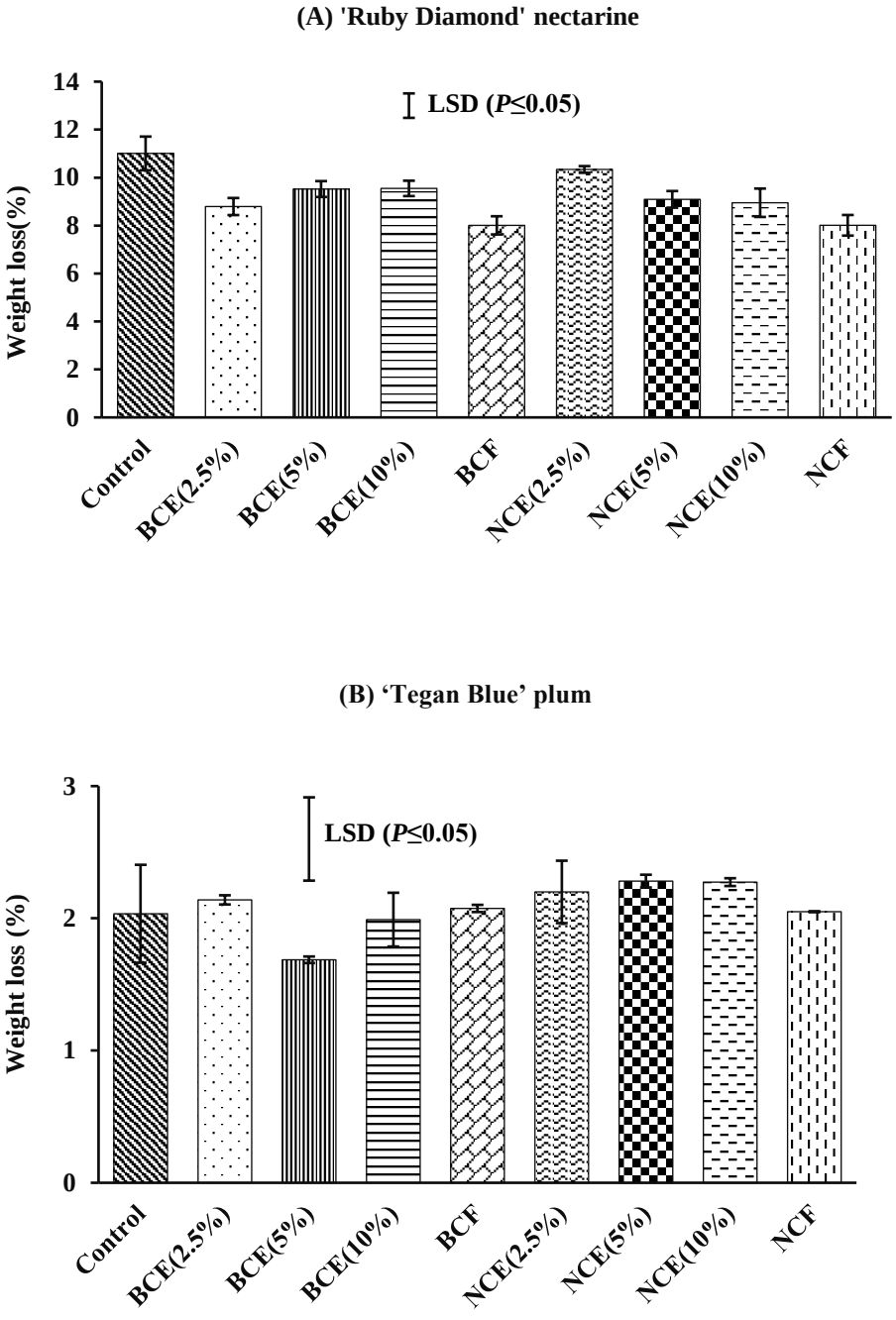

Fig. 7. 5 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on weight loss (%) of 'Ruby Diamond' nectarine (A) and 'Tegan Blue' plum nectarine (B) after 25 d cold storage at 1°C. Vertical bars represent SE of means of three replicates and are not visible when the values are too small.

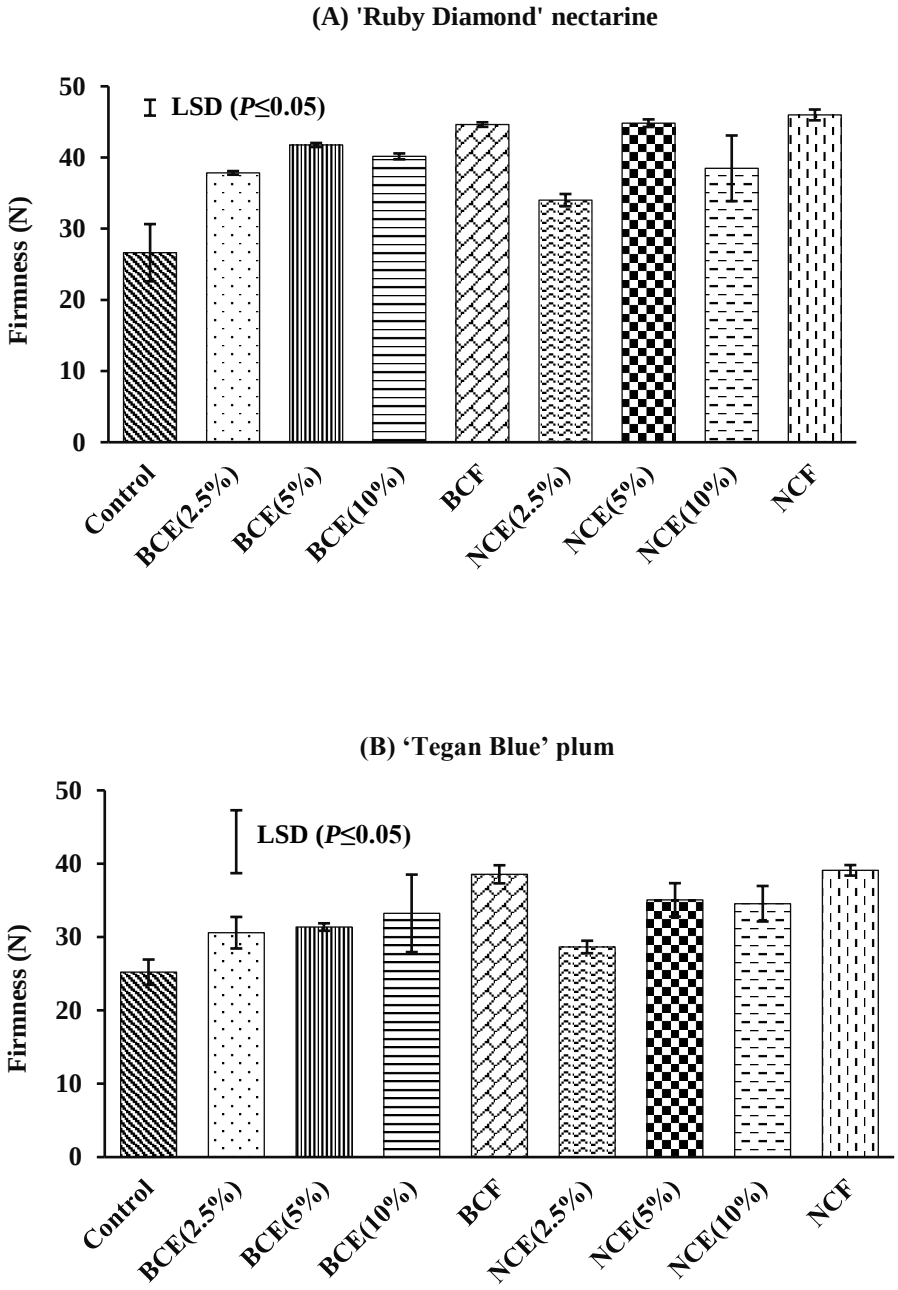

Fig. 7. 6 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on firmness (N) of 'Ruby Diamond' nectarine (A) and 'Tegan Blue' plum nectarine (B) after 25 d cold storage at 1°C. Vertical bars represent SE of means of three replicates and are not visible when the values are too small.

7.3.4 SSC, TA and SSC:TA

The soluble solids content of ‘Ruby Diamond’ nectarine treated with BC and NC, irrespective of the formulations, were significantly lower as compared to control after 25 d cold storage (Table 7.5). The level of SSC in the nectarine fruit treated with BC aqueous solution 5% ethanol was recorded as the lowest (13.7%), followed by the fruit fumigated with BC (13.7%). In ‘Tegan Blue’ plum, the fruit treated with NC aqueous solution with 5% ethanol showed the highest SSC percent (13.3%) followed by the fruit fumigated with NC (13.6%).

TA of the treated and untreated ‘Ruby Diamond’ nectarines were not significantly different from each other, whilst TA of ‘Tegan Blue’ plum treated with BC and NC formulations were significantly reduced ≈1.1 fold as compared to control (Table 7.5). The plum fumigated with NC exhibited the highest level of TA (1.7%) as compared to control and the rest of the treated fruit.

Similar to TA content, the ratio of SSC:TA in ‘Ruby Diamond’ nectarine was not significantly affected by the ethylene antagonists (Table 7.5). However, SSC:TA of ‘Tegan Blue’ plums treated with BC fumigation, NC fumigation and BC aqueous solution with 5% ethanol were significantly reduced up to 1.1 fold when compared to control after 25 d cold storage.

7.3.5 Individual sugars

After 25 d storage at 1°C, glucose, sucrose, fructose and sorbitol were identified as individual sugars in ‘Ruby Diamond’ nectarine and ‘Tegan Blue’ plum (Table 7.6). Sucrose was observed to be the predominant sugar in ‘Ruby Diamond’ nectarine while fructose was the predominant one in ‘Tegan Blue’ plum. The levels of glucose in the nectarine fruit treated with fumigations of BC and NC, aqueous solutions of BC with 10% ethanol and NC with 5% ethanol were maintained significantly lower up to 1.2 fold as compared to control and rest of the treatments. Sucrose levels of ‘Ruby Diamond’ nectarine treated with NC formulations, except NC aqueous solution with 10% ethanol, and BC fumigation were retained higher ≈1.1 fold as compared to control. However, in ‘Tegan Blue’ plum, the fruit treated with BC and NC, except NC aqueous solution with 10% ethanol exhibited significantly higher (1.2-1.5 fold) sucrose concentrations as compared to control. The level of sucrose in the plums

fumigated with BC and NC were recorded to be the highest (49.96 % and 49.72 %, respectively) amongst all the treatments. The level of fructose was not affected by the ethylene antagonists in both tested nectarines and plums. The level of sorbitol in the nectarine fruit treated with NC aqueous solution with 2.5% ethanol was 1.3 fold higher than control. In contrast, the level of sorbitol in the plum fruit treated with NC aqueous solution with 5% ethanol was 1.3 fold lower as compared to control.

7.3.6 Individual organic acids

Malic, succinic, fumaric and citric acids were identified in 'Ruby Diamond' nectarine and 'Tegan Blue' plum stored for 25 d at 1°C (Table 7.7). Malic acid concentrations of 'Ruby Diamond' nectarine treated with BC and NC fumigations and aqueous solutions of NC with 5% ethanol were significantly higher as compared to control. The levels of malic and citric acid in 'Tegan Blue' plum and the levels of succinic acid in 'Ruby Diamond' nectarine treated with different formulations of BC and NC were not significantly different from control fruit. The concentration of fumaric acid in the nectarine fruit treated with NC, except aqueous solution with 2.5% ethanol, were maintained significantly higher as compared to control. Similarly, the plum treated with aqueous solutions of BC and NC, regardless of ethanol concentration, exhibited significantly higher fumaric acid concentration (≈1.1 fold) as compared to control after 25 d cold storage.

7.3.7 Total phenols, ascorbic acid and antioxidant capacity

Total phenolic content of 'Tegan Blue' plum fumigated with NC was significantly lower (1.2 fold) than control (Table 7.8). However, it was not significantly different from the plum treated with NC aqueous solutions with 2.5% and 5% ethanol, BC aqueous solution with 5% ethanol and BC fumigation. The contents of total phenols in the 'Ruby Diamond' nectarine treated with BC and NC were not significantly different as compared to control (Table 7.8). Similarly, ascorbic acid and total antioxidant capacity of the 'Ruby Diamond' and 'Tegan Blue' plum treated with different formulations of BC and NC were also not significantly different to control fruit after 25 d cold storage (Table 7.8).

Table 7. 5 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on SSC (%), TA (%) and SSC:TA ratio of 'Ruby Diamond' nectarine and 'Tegan Blue' plum after 25 d at 1°C.

Treatments	**SSC (%)**		**TA (%)**		**SSC:TA**	
	Ruby Diamond	**Tegan Blue**	**Ruby Diamond**	**Tegan Blue**	**Ruby Diamond**	**Tegan Blue**
Control	15.7±0.1e	14.6±0.00f	1.1±0.2	1.34±0.0a	13.9±2.2	10.9±0.1f
BCE (2.5%)	14.1±0.1abc	14.4±0.00e	1.1±0.1	1.56±0.02d	14.0±0.9	9.22±0.1d
BCE (5%)	13.7±0.1a	14.1±0.03d	1.2±0.1	1.65±0.02e	12.8±0.6	8.80±0.1bc
BCE (10%)	14.8±0.1d	14.5±0.03f	1.2±0.1	1.54±0.0cd	11.5±0.7	9.17±0.02cd
BCF	13.7±0.1ab	13.8±0.05c	1.2±0.2	1.63±0.02e	12.4±1.4	8.45±0.1b
NCE (2.5%)	14.3±0.4acd	14.0±0.03d	1.1±0.1	1.45±0.02b	13.2±0.4	9.67±0.1e
NCE (5%)	14.2±0.1abcd	13.3±0.03a	1.2±0.04	1.52±0.02bcd	12.3±0.5	8.74±0.02b
NCE (10%)	14.6±0.03cd	14.1±0.0d	1.1±0.1	1.47±0.03bc	13.6±0.9	9.58±0.2de
NCF	14.8±0.1d	13.6±0.03b	1.1±0.04	1.70±0.02e	13.6±0.4	8.0±0.1a
LSD ($P \leq 0.05$)	0.5472 **	0.09 **	ns	0.067 **	ns	0.382 **

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Ruby Diamond' and 'Tegan Blue' are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 7. 6 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on individual sugar and sugar-acid contents of 'Ruby Diamond' nectarine and 'Tegan Blue' plum after 25 d at 1°C.

Treatments	Individual sugars and sugar-acid (g kg^{-1})							
	Glucose		Sucrose		Fructose		Sorbitol	
	Ruby Diamond	Tegan Blue	Ruby Diamond	Tegan Blue	Ruby Diamond	Tegan Blue	Ruby Diamond	Tegan Blue
Control	18.8±0.3d	42.58±2.5	104.0±1.9ab	33.34±3.4a	34.6±0.3	58.73±1.9	8.7±0.3ab	38.88±3.2bc
BCE (2.5%)	18.9±0.7d	35.00±0.3	102.0±1.3ab	42.39±0.3bcd	32.3±0.2	45.80±0.5	9.3±0.1ab	37.03±0.2ab
BCE (5%)	17.9±0.2cd	36.49±1.3	106.7±1.9abc	46.21±1.8cde	31.7±0.7	51.02±2.4	8.7±0.1ab	36.12±1.9ab
BCE (10%)	17.4±0.3abc	40.20±1.6	100.1±2.6a	47.91±0.9de	31.6±1.3	56.73±2.7	7.8±0.3a	36.84±0.8ab
BCF	16.5±0.1ab	40.09±1.5	112.2±4.8bc	49.96±1.5e	30.7±1.0	55.98±2.5	7.6±0.2a	39.68±1.6bc
NCE (2.5%)	18.3±0.3cd	40.67±1.1	117.5±4.8c	43.98±1.1bcde	34.2±1.7	55.98±2.0	10.9±1.2c	39.34±1.8bc
NCE (5%)	17.0±0.2abc	39.85±2.5	115.3±1.9c	40.69±0.5bc	31.3±0.2	50.90±0.9	10.2±0.02bc	31.16±0.8a
NCE (10%)	17.6±0.3bcd	42.29±1.9	101.9±1.3ab	38.75±2.5ab	31.5±0.4	52.65±3.7	9.1±0.2ab	34.85±2.8ab
NCF	16.2±0.2a	37.58±0.5	112.8±0.7bc	49.72±1.9e	31.2±0.1	55.80±4.6	7.7±0.1a	44.10±1.7c
LSD ($P \leq 0.05$)	1.218 *	ns	10.21 *	6.138 **	ns	ns	1.578 *	5.967 *

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Ruby Diamond' and 'Tegan Blue' are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 7. 7 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on individual organic acid contents of 'Ruby Diamond' nectarine and 'Tegan Blue' plum after 25 d at 1°C.

Treatments	Individual organic acids (g kg^{-1})							
	Malic		Succinic		Fumaric		Citric	
	Ruby Diamond	Tegan Blue	Ruby Diamond	Tegan Blue	Ruby Diamond	Tegan Blue	Ruby Diamond	Tegan Blue
Control	4.3±0.04ab	18.94±0.3	2.6±0.1	3.8±0.2	0.41±0.0bcd	0.33±0.0a	6.95±0.2	0.33±0.03
BCE (2.5%)	4.2±0.2ab	20.86±1.6	2.4±0.1	3.6±0.1	0.41±0.0a	0.34±0.0b	6.58±0.3	0.33±0.01
BCE (5%)	5.2±0.3abc	21.70±0.7	2.5±0.02	3.8±0.2	0.41±0.0ab	0.34±0.0b	6.97±0.02	0.36±0.01
BCE (10%)	5.5±0.7bcd	23.82±0.8	2.4±0.02	4.1±0.2	0.41±0.0bc	0.35±0.0b	6.34±0.1	0.37±.0.03
BCF	6.8±0.6de	23.57±0.9	2.3±0.04	4.1±0.2	0.42±0.0cde	0.33±0.0a	6.28±0.3	0.37±0.01
NCE (2.5%)	3.9±0.1a	22.79±0.6	2.4±0.1	4.1±0.1	0.42±0.0def	0.34±0.0b	6.35±0.2	0.38±0.01
NCE (5%)	6.2±0.7cde	21.66±0.1	2.4±0.1	4.4±0.2	0.42±0.0f	0.36±0.0c	6.16±0.03	0.34±0.01
NCE (10%)	3.8±0.1a	21.26±1.2	2.4±0.1	3.9±0.1	0.42±0.0ef	0.35±0.0b	5.97±0.1	0.34±0.01
NCF	7.4±0.1e	23.16±0.2	2.3±0.01	4.3±0.1	0.42±0.0f	0.32±0.0a	6.18±0.03	0.35± 0.01
LSD ($P \leq 0.05$)	1.403 **	ns	ns	ns	0.002 **	0.011 **	ns	ns

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Ruby Diamond' and 'Tegan Blue' are independent from each other. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 7. 8 Effect of BC and NC aqueous solutions with ethanol (2.5, 5 and 10%), and fumigations on total phenols, ascorbic acid and total antioxidant capacity of 'Ruby Diamond' nectarine and 'Tegan Blue' plum after 25 d at 1°C.

Treatments	**Total phenols (g GAE kg^{-1})**		**Ascorbic acid (g kg^{-1})**		**Total antioxidants capacity (mmol TEAC kg^{-1})**	
	Ruby Diamond	**Tegan Blue**	**Ruby Diamond**	**Tegan Blue**	**Ruby Diamond**	**Tegan Blue**
Control	32.6±1.8	79.3±4.8bc	9.21±0.4	21.6±1.4	59.4±1.9	181.7±3.1
BCE (2.5%)	29.9±0.4	79.5±3.3bc	8.04±0.3	20.7±0.3	69.5±3.7	178.8±4.6
BCE (5%)	22.3±1.3	69.9±2.8ab	8.39±0.6	20.6±0.1	61.0±0.8	186.8±4.0
BCE (10%)	30.2±3.3	71.4±4.0ab	8.37±0.4	20.9±0.3	51.9±3.2	186.4±3.3
BCF	22.5±2.0	76.5±3.7abc	8.07±0.2	19.3±0.3	53.3±0.5	185.1±2.6
NCE (2.5%)	30.7±2.5	69.5±1.8ab	8.81±0.2	19.0±0.4	54.6±0.8	162.4±7.0
NCE (5%)	28.8±2.4	84.8±3.8c	7.90±0.5	20.4±0.1	61.3±3.4	185.7±7.0
NCE (10%)	34.7±4.4	71.9±0.8ab	10.54±0.9	18.8±0.3	71.5±3.8	191.7±2.4
NCF	32.6±6.0	68.0±2.8a	8.67±0.4	20.3±0.5	65.7±7.3	192.2±7.7
LSD ($P \leq 0.05$)	11.14	10.03 *	ns	ns	ns	ns

Means in the same column followed by the different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. The letterings for 'Ruby Diamond' and 'Tegan Blue' are independent from each other.

7.4 Discussion

7.4.1 Ethylene production

Ethylene production of untreated and treated 'Ruby Diamond' nectarine and 'Tegan Blue' plum fruits were checked daily for 8 d during the ripening period after 0 d and 25 d cold storage at 1°C. The plum fruit treated BC and NC, except aqueous solution of BC with 5% ethanol, and aqueous solutions of BC and NC with 10% ethanol, exhibited a reduced rate of climacteric ethylene production. In nectarine fruit, only BC and NC fumigation treatments were effective in reducing the climacteric ethylene production. According to Sisler et al. (2006), the ethylene antagonists bind to the ethylene receptors in the plant tissues and inactivate the ethylene signal response. Pirrung et al. (2008) have revealed that the receptor-binding mechanism of ethylene antagonist 1-methylcyclopropene is though the ring-opening reaction. The present ethylene antagonists, 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) contain a cyclopropene fused to a benzene ring which reacts in a similar mechanism (Halton, 1973). Therefore, the receptor binding mechanism is likely to be the same as 1-MCP in inhibiting the ethylene action and thus, in turn, suppressing the ethylene production. 1-MCP (1 or 2 µL L^{-1}) reduced the ethylene production of 'Tegan Blue' plum stored for 17 d at 20±1°C or stored for 0, 3 and 6 weeks at 0±1°C and 90±5% RH (Khan and Singh, 2007a and 2009). In the present study, the effectiveness of BC and NC fumigation on the suppression of climacteric ethylene production in 'Ruby Diamond' nectarine was more pronounced when they were kept under ambient conditions. This is consistent with the observations reported by Bregoli et al. (2005) that showed 1 µL L^{-1} of 1-MCP suppressed the ethylene production of 'Stark Red Gold' nectarine depended on the storage temperature. They observed that the ethylene production of 1-MCP treated nectarine was effectively reduced when they are stored at 25°C.

The plum fruit treated with aqueous solutions of BC and NC, except with 10% ethanol, exhibited a significant reduction in climacteric ethylene production. This can be attributed to the presence of ethanol (at the concentration of 2.5 or 5.0 %) which helps to enhance the performance of BC and NC by improving the delivery through the fruit

cuticle. Similarly, improved performance of active compound (ethephon) by the presence of ethanol has been reported in Cranberry (Farag et al., 1992). However, the presence of ethanol at higher concentration, i.e. 10% concentration of ethanol in the present study, might have totally disrupted the barrier properties of the fruit cuticle which, according to Lara et al. (2014), can decrease the postharvest storage life of fruit.

7.4.2 Respiration rate

The onset of climacteric respiration peak of 'Tegan Blue' plum treated with BC and NC were delayed up to 2 d, although the respiration rate was not significantly different, as compared to control after 0 d storage at 1°C. Similar delayed onset of climacteric respiration peak was reported in the 'Royal Zee' plum treated with 1000 nL L^{-1}1-MCP stored for 10 d at 0 °C (Dong et al., 2002). The climacteric respiration peak and peak rate of the 'Ruby Diamond' nectarine were not affected by the ethylene antagonists in both storage conditions, which is consistent with the previous findings in 'Almog' and Oded' peaches and 'April Glow' nectarine (Liguori et al., 2004).

7.4.3 Weight loss and firmness

Weight loss and fruit firmness, the important fruit ripening quality indices influenced by ethylene, were also significantly affected by the BC and NC aqueous solutions. The significant reduction in physiological weight loss was observed in 'Ruby Diamond' nectarine fruit fumigated with BC and NC. The fruit weight loss of 'Tegan Blue' plum fumigated with BC and NC were comparatively lower, although they were not significantly different from the control. Similar weight loss reduction by fumigation with ethylene antagonist was previously reported in 'Santa Rosa' and 'Golden Japan' plums treated with 0.25, 0.50 or 0.75 µL L^{-1} of 1-MCP at 1°C (Martinez-Romero et al., 2003).

Fruit firmness was substantially higher in both nectarine and plum fumigated with BC and NC, and in nectarine treated with BC and NC aqueous solutions. The firmness retention might have been obtained by blocking the action of ethylene which is the key promoter of fruit softening enzyme activities in plums and nectarines (Liguori et al., 2004; Khan and Singh, 2007a). The higher firmness retention after ethylene antagonist

treatment during cold storage was reported in ‘Tegan Blue’ plum (Khan and Singh, 2009), in ‘Harrow Sun’ plum (Manganaris et al., 2007) and in ‘Stark Red Gold’ nectarine stored at 25 °C (Bregoli et al., 2005).

7.4.4 SSC, TA and SSC:TA

BC and NC irrespective of the application method maintained a significantly lower level of SSC in both tested nectarine and plum. Higher TA percent and lower SSC:TA ratio were observed in ‘Tegan Blue’ plum treated with BC and NC while the TA percent and the SSC:TA ratio were not affected by BC and NC. The retention of lower SSC and higher TA percent can be ascribed to the result of the delayed ripening process driven by BC and NC. This result is consistent with the reduction in soluble solids content reported for 1-MCP-treated ‘Maria Aurelia’ nectarine (Ozkaya et al., 2016) and in ‘Songold’ plum (Velardo-Micharet et al., 2017). Similarly, higher retention of titratable acidity in 1-MCP treated fruit was reported in ‘Harrow Sun’ plums stored under different storage temperature (Manganaris et al., 2007).

7.4.5 Individual sugars and organic acids

Among the identified individual sugars, sucrose was found to be the major sugar in ‘Ruby Diamond’ nectarine, whilst fructose was the predominant one in ‘Tegan Blue’ plum. Similar findings were reported in different peach cultivars (Saidani et al., 2017), in nectarines (Abdi et al., 2011) and in Japanese plums (Singh et al., 2009). The response of individual sugars and organic acids to the ethylene antagonists were varied depending on the fruit type and the application method. The higher retention of sucrose and malic acid contents in the ‘Ruby Diamond’ nectarine and ‘Tegan Blue’ plum treated with NC aqueous solutions with 2.5 or 5 % ethanol, treated with BC and NC, except NC aqueous solution with 10 % ethanol may be due to the slow ripening processes which in turn delay the sugar metabolism. Similarly, the substantially higher amount of malic acid in the ‘Ruby Diamond’ nectarine treated with BC and NC fumigations, and NC aqueous solution with 5% ethanol indicated the delayed degradation of malic acids. According to Borsani et al., (2009), sucrose concentration was gradually decreased with the increase in glucose and fructose accumulation during

fruit ripening (within 7 d after harvest at 20°C). They also reported that organic acid accumulation was higher during fruit development and gradually decreased along with the commencement of ripening. It has been reported that the application of 1-MCP at the concentration of 2 µL L^{-1} for 24 h at 5°C delayed the metabolic break down of sucrose in the 'Gold Nijisseiki' and 'Hosui' pears (Itai and Tanahashi, 2008). Similar delayed metabolic breakdown of the organic acids was reported in 'Fuji' apple treated with 1 µL L^{-1} of 1-MCP for 24 h at 20°C (Liu et al., 2016). In contrast, 'Stark Red' nectarine treated with 1 µL L^{-1} 1-MCP for 12 h and stored at 4°C for 3 d did not show any significant difference in sugar and organic acid contents as compared to control (Bregoli et al., 2005). These variations in response may be due to differences in the effectiveness of 1-MCP depending on the fruit type or the concentration of 1-MCP applied as reported by Dong et al. (2002) in 'Canino' apricot and 'Royal Zee' plum.

7.4.6 Total phenols, ascorbic acid and total antioxidant activity

'Tegan Blue' plum exhibited relatively higher contents of total phenols, ascorbic acid and total antioxidant activity than 'Ruby Diamond' nectarine. The varietal dependent variation of phytochemical compounds and the antioxidant activities were formerly reported in different varieties of peaches, nectarines and plums (Gil et al., 2002). The lower retention of total phenols in 'Tegan Blue' plum fumigated with NC may be attributed to the reduction in ethylene production which was induced by the ethylene antagonist. Ethylene positively regulates the activity of phenylalanine ammonia lyase enzymes which is related to the biosynthesis of phenolic compounds (Blankership and Unrath, 1987). A similar reduction of total phenolic contents was reported in 'Yuhualu' peach fruit treated with 5 µL L^{-1} of 1-MCP for 24 h and stored at 20°C for 10 d (Liu et al., 2015). In the present study, ascorbic acid and total antioxidant activity of the tested nectarine and plum fruit did not respond to BC and NC. Similarly, Khan and Singh (2008) reported that the ascorbic acid contents and the total antioxidant activity of untreated and treated 'Tegan Blue' plum with 1.0 µL L^{-1} of 1-MCP for 24 h did not show any significant changes after 15, 30, 45 and 60 d cold storage at 0±1°C. However, contrasting findings were reported in 'Yuhualu' peach fruit treated with 5 µL L^{-1} of 1-MCP for 24 h and stored at 20°C for 10 d (Liu et al., 2015), where 1-MCP application maintained higher ascorbic acid content and postponed the intensification

of antioxidant activity as compared to control. These differences in the effect of 1-MCP may be ascribed to differences in the species, the treatment concentrations, the storage temperature and duration.

7.5 Conclusion

The effect of aqueous solutions of BC and NC on climacteric ethylene production were varied depending on the fruit species, ethanol concentration and the storage conditions. The aqueous solutions of BC and NC prepared with 2.5% or 5% ethanol were found to be the most effective in suppressing the climacteric ethylene production of 'Tegan Blue' plum stored for 25 d at 1°C. The use of ethanol at high concentration (10%) accelerated ethylene production, which is due to the disruption of fruit cuticle. The fumigation with BC and NC substantially suppressed the climacteric ethylene production of 'Ruby Diamond' nectarine and 'Tegan Blue' plum after 0 and 25 d storage at 1°C. The differences in the response of fruit firmness, SSC, TA, SSC:TA ratio, individual sugars and organic acids to the BC and NC were also observed depending on the fruit species and the formulations applied. The responses of fruit quality parameters to BC and NC aqueous solutions prepared with 5% ethanol were in line with the response of ethylene production. BC and NC did not exhibit any significant effect on the level of phytochemical compounds in both nectarine and plum.

CHAPTER 8

Effectiveness of ethylene antagonists (BC, NC and 1-MCP) and modified atmosphere packaging on ethylene production and postharvest fruit quality of plum and pluot under cold storage

Summary

The effect of ethylene antagonists and modified atmosphere packaging (MAP) in prolonging cold storage life and maintaining fruit quality of plum and pluot was investigated. Two separate experiments were conducted on 'Angeleno' plum and 'Flavor Fall' pluot fruits. In the first experiment, plum fruit were treated with 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) fumigations (1 µM) or spray solutions (2 µM) and stored with or without MAP for 35 and 50 d at 0±1 °C and 90±5% RH. In the second experiment, the pluot fruit were treated with BC, NC and 1-methylcyclopropene (1-MCP) fumigations or BC and NC spray solutions, and stored with or without MAP for 40 and 60 d at 0±1 °C and 90±5% RH. The ethylene antagonists and MAP significantly suppressed and delayed the climacteric ethylene production of plum and pluot, except in 'Angeleno' plum cold-stored for 50 d. The effects of BC and NC spray solutions on the climacteric ethylene peak onset and concentration of plum and pluot were varied depending on fruit type and cold storage period. The effectiveness of BC and NC spray solutions on ethylene production was more pronounced in combination with MAP. Regardless of MAP, the fumigation with BC, NC and 1-MCP exhibited significant effect in maintaining the fruit firmness, soluble solids content, sugars and organic acid contents. The weight loss was substantially reduced in the plum and pluot fruits stored with MAP. The antioxidant activity and the levels of total phenols and ascorbic acid of the plum and pluot treated with ethylene antagonists, irrespective of MAP, were significantly the same as of control fruit. The interaction effect between the ethylene antagonists and MAP on ethylene production and postharvest fruit quality were different in plum and pluot depending on cold storage period. In conclusion, the fumigation with ethylene antagonists with or without MAP, can reduce ethylene production and maintain fruit quality of 'Angeleno' plum and 'Flavor Fall' pluot up to respective 50 and 60 d. The aqueous solutions of BC and NC performed better with the presence of MAP in ethylene suppression and quality maintenance.

8.1 Introduction

The Prunus species, plum and pluot, are commercially important stone fruits with high perishability (Hummer and Janick, 2009). Pluot is the interspecific cross between plum and apricot, which has a greater percentage of plum characters (Crisosto et al., 2007). Plums are commonly considered as climacteric fruit with a distinct rise in internal ethylene production during ripening. There are also suppressed-climacteric plum types such as 'Rubyred', 'Shiro' and 'Late Santa Rosa'plums which do not exhibit a clear climacteric ethylene peak as the climacteric cultivars do during ripening (Abdi et al., 1997 and 1998; Minas et al., 2015). However, these suppressed-climacteric plum cultivars, like other climacteric plums, are also sensitive to ethylene (Manganaris et al., 2008).

Ethylene is a natural plant hormone which regulates fruit ripening and ripening related biochemical processes in fruits (Burg and Burg, 1965), including plums (Abdi et al., 1997). Even at very low levels of 0.01-0.1 µL $kg^{-1}h^{-1}$, ethylene can promote ripening process and can induce physiological disorders such as mealiness and internal breakdown of plums (Crisosto and Kader, 2000). In response to the exogenously applied ethylene, the climacteric plum 'Joanna Red' and the suppressed-climacteric plum 'Angeleno' showed an acceleration in a range of ripening processes such as increased ethylene production and respiration rate, quick reduction in fruit firmness and titratable acidity (Minas et al., 2015). Due to the high sensitivity to ethylene, plums have a very short storage life with limited export market potential (Khan et al., 2018).

Cold storage is a commonly practised technology to delay fruit ripening and to maintain the postharvest fruit quality of plums (Mitchell et al., 1974; Manganaris et al., 2008). Modified atmospheric packaging (MAP) is an alternative way to extend the storage life of fruits by maintaining the atmospheric condition of high carbon dioxide and low oxygen concentrations. This modified atmosphere is created through the respiration of the fruit where oxygen is depleted as respiratory substrate and carbon dioxide is produced as a respiratory product (Valero and Serrano, 2010b). With the application of MAP, the 'Friar' plum can be stored for 45 days at 0 °C and 85 % RH (Cantin et al., 2008) and yellow plums for 20 ds at 1±1 °C and 90-95% RH (Sottile et al., 2013). The ethylene antagonist 1-MCP has been demonstrated as an effective treatment to regulate plum fruit ripening while maintaining postharvest fruit quality

either as fumigation (Khan and Singh, 2007a) or as dip solution (Manganaris et al., 2007). It has also been reported that the combined application of 1-MCP and MAP can extend the cold storage life (3-5 weeks) of 'Tegan Blue' plum to 7 weeks by suppressing the activities of enzymes responsible for ethylene biosynthesis and fruit softening (Khan and Singh, 2008).

The recently developed ethylene antagonists, 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) were observed to be effective in delaying fruit ripening of plums by blocking the ethylene action (Singh et al., 2018). The effect of BC and NC on postharvest fruit quality of pluot is yet to be investigated. The synergistic effect of these ethylene antagonists (BC and NC) and MAP on ethylene production and postharvest fruit quality of plum and pluot is still unknown. Therefore, the effect of ethylene antagonists (BC, NC and 1-MCP) alone or in combination with MAP to extend the cold storage life and to maintain the postharvest fruit quality of plum and pluot was investigated. It was hypothesised that the application of ethylene antagonists and MAP will improve the cold storage life of plum and pluot while preserving fruit quality.

8.2 Materials and methods

8.2.1 Experimental conditions

Two independent experiments were conducted to investigate the effect of ethylene antagonists (BC, NC and 1-MCP) in combination with or without modified atmosphere packaging (MAP) on extending the cold storage life of plum and pluot. The fruit were sourced from the commercial farms as mentioned in section 3.1. The information on tree age, rootstock and the plant spacing for the tested cultivars were mentioned in Chapter 3, Table 3.1. The supplier of ethylene antagonists was mentioned in Chapter 3, Section 3.2 and the chemical name, formula, chemical structure and the molecular weight of BC, NC and 1-MCP were detailed in Chapter 3, Table 3.2. The preparation of spray solutions and the fumigation treatments were done following the procedure of Choi et al., (2008) and Khan (2007) with some modifications as described in Chapter 3 section 3.3.1. The adjuvants Tween® 20 (0.02%) and ethanol (5%) were

applied in the preparation of BC and NC spray solutions to enhance the performance of BC and NC in inhibiting the ethylene action.

8.2.1.1 Experiment 1: Effect of BC and NC in combination with or without MAP on extending the storage life of 'Angeleno' plum

'Angeleno' plums (*Prunus salicina* L.) from the Eastwind orchard were sourced on 1st March 2018. Fruit without any mechanical defects were selected to use as samples. The fruit were distributed and applied with the respective treatments as described in Fig. 8.1. For the treatments with BC and NC spray solutions, the respective fruit sample were sprayed thoroughly with the freshly prepared BC and NC solutions using a 500 mL hand sprayer. For the treatments with BC and NC fumigation, the fruit were fumigated using a 60 L air-tight container. Along with the sample fruit, soda-lime was kept to avoid the accumulation of carbon dioxide inside the container. A portable fan was also placed to generate an equal amount of BC and NC vapour inside the container. Treatment application was carried out at 20±1°C and the treated fruit were kept in the air-tight plastic containers for 18 h. After 18 h, the fruit were taken out of the containers in an open space and arranged by replication in the cardboard boxes which were labelled accordingly to the treatments, storage type and storage period (as in Fig. 8.1). After that, the labelled boxes were stored at 0±1°C and 90±5% RH for 35 d and for 50 d. For all the treatments with modified atmospheric packaging (MAP), the treated fruit were kept in the commercial Life span® MAP bags and kept for 4 h at 1°C. The bags were sealed tightly after 4 h to maintain the balanced temperature inside and outside of the bag (Khan and Singh, 2008). The physiological parameters (ethylene production, respiration rate, weight loss and fruit firmness) and fruit quality parameters (SSC, TA, SSC:TA, individual sugars and organic acids, total phenols, ascorbic acid and antioxidant capacity) were checked after 35 and 50 d cold storage as mentioned in Section 8.2.2.

8.2.1.2 Experiment 2: Effect of BC and NC in combination with or without MAP on extending the storage life of 'Flavor Fall' pluot

'Flavor Fall' pluots (*P. salicina* x *(P. salicina* x *P. armeniaca)* from the Della Pollina orchard were sourced on 24th March 2018. The same experimental procedure as in experiment 1 was applied using BC, NC and 1-MCP, except for the cold storage periods (40 and 60 d). The fruit distribution and the treatments were described in the following Fig. 8.2. The 1-MCP fumigation treatment was applied following the same procedure of BC and NC fumigation treatments in experiment 1.

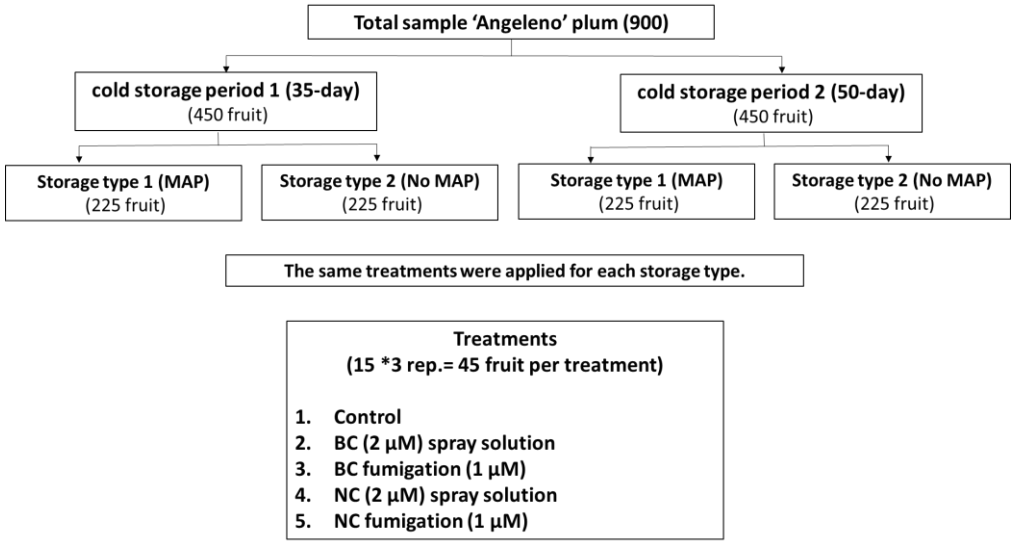

Fig. 8. 1 Sample fruit distribution and the treatments for experiment 1.

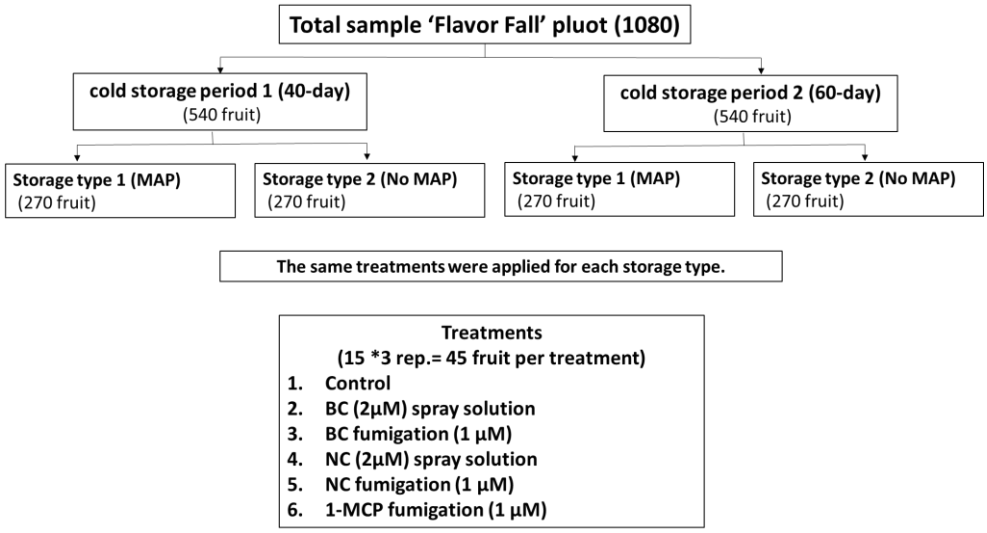

Fig. 8. 2 Sample fruit distribution and the treatments for experiment 2.

8.2.2 Determination of physiological parameters

8.2.2.1 Ethylene production (nmol kg^{-1} h^{-1})

Ethylene production of 'Angeleno' plum and 'Flavor Fall' pluot were determined at 20°C for 10 d and 8 d, respectively (Fig. 8.3 and 8.4) after removal from cold storage. Three representative fruit per replication of 'Angeleno' plum and 'Flavor Fall' pluot were checked for ethylene production using the laser-based ethylene detector ETD-300 following the method of Azzu (2016). The detailed function of ETD-300 and the monitoring procedure of ethylene are explained in Chapter 3, Section 3.8.1 and the ethylene production is described as nmol kg^{-1} h^{-1}.

8.2.2.2 Respiration rate (mmol CO_2 kg^{-1} h^{-1})

The same 'Angeleno' plums and 'Flavor Fall' pluots selected to determine ethylene production were used to determine the respiration rate. The respiration rate was analysed as CO_2 production by using a gas analyser following the detailed procedure of Khan and Singh (2008). The detailed procedure for estimation of respiration rate is explained in Chapter 3, Section 3.8.2 and the rate of respiration is described as mmol CO_2 kg^{-1} h^{-1}.

8.2.2.3 Physiological weight loss (%) and fruit firmness (N)

The initial and final weight of each replication were checked before and after cold storage. The final weight was subtracted from the initial weight to calculate the percent physiological weight loss according to the formula previously described by Azzu (2016). The detailed calculation is described in Chapter 3, Section 3.8.3.

Twelve representative fruit per replication was used to determine fruit firmness. Firmness was measured two times for each fruit by using a texture analyser fitted with 8 mm probe following the method previously described by Khan and Singh (2008). The detailed procedure is mentioned in Chapter 3, Section 3.8.8 and the firmness is reported as N.

8.2.3 Determination of quality parameters

8.2.3.1 SSC (%), TA (%) and SSC:TA

The same fruit used for firmness determination were also used to quantify the levels of SSC, TA and SSC:TA ratio following the method of Khan and Singh (2008). A slice of pulp was taken from each fruit to prepare the juice. From the freshly prepared juice, the percent soluble solids content was measured using a portable digital refractometer. The malic acid equivalent TA (%) was determined by titrating the juice sample with 0.1 N NaOH using phenolphthalein as an indicator. The detailed quantification procedures for SSC (%), TA (%) and SSC:TA ratio were described in Chapter 3, Section 3.6.1.

8.2.3.2 Individual sugars and organic acids (g kg^{-1})

Individual sugars and organic acids were quantified by following the detailed procedure of Usenik et al. (2008) with some modifications using a high-performance liquid chromatography (HPLC). Glucose, fructose, sucrose and sorbitol as individual sugar and sugar-alcohol, and malic, citric, fumaric and succinic acids as individual organic acids were identified using the respective standard sugars and acids. The sample preparation and quantification procedures were mentioned in detail in Chapter 3, Section 3.6.2. The individual sugars and organic acids were reported as g kg^{-1} fresh pulp weight.

8.2.3.3 Total phenols (g GAE kg^{-1})

The total phenolic content was quantified following the procedure previously described by Cantin et al. (2009) with little modifications. The extraction procedure and assessment protocol for the total phenolic contents were detailed in Chapter 3, Section 3.6.3. The absorbance was measured at the wavelength of 750 nm using a spectrophotometer and the total phenolic content was quantified as gallic acid equivalents gram per kilogram (g GAE kg^{-1}) of fresh pulp weight.

8.2.3.4 Ascorbic acid (g kg^{-1})

The estimation of ascorbic acid content was undertaken by following the procedure of Vithana (2017). The quantification of ascorbic acid was done at the wavelength of 760

nm using a spectrophotometer. The protocol for ascorbic acid extraction and assessment were mentioned in detail in Chapter 3, Section 3.6.8 and the ascorbic acid content was expressed as g kg^{-1}.

8.2.3.5 Total antioxidant capacity (mmol TEAC kg^{-1})

The free radical scavenging capacity of the pulp was checked to determine the total antioxidant capacity by the DPPH method as previously described by Vithana (2017) based on Brand-Williams et al. (1995). The protocol for standard free radical solution preparation, antioxidant extraction and quantification were detailed in Chapter 3, Section 3.6.5. The absorbance values were checked until they reached the range of 0.6-0.7 using a spectrophotometer at the wavelength of 515 nm. The total antioxidant capacity of the pulp sample was determined by standardising with the Trolox standard curve and expressed as mM Trolox equivalent per kg (mmol TEAC kg^{-1}).

8.2.4 Statistical analysis

The recorded data were analysed by using Genstat software as mentioned in previous chapters. The treatment effects were compared by calculating the least significant differences (LSD) at ($P \leq 0.05$). The data were subjected to a two-way analysis of variance (treatments x storage type). The multiple mean comparison was calculated by using Duncan's multiple range test. The data for each cold storage period of 'Angeleno' plum and 'Flavor Fall' pluot were analysed individually.

8.3 Results

8.3.1 Ethylene production

Ethylene production of 'Angeleno' plum was in the range of 0.05-0.11 nmol kg^{-1} h^{-1} (Fig. 8.3). After 35 d cold storage, the ethylene antagonists BC and NC, regardless of the treatments, significantly delayed the ethylene peak onset of 'Angeleno' plum as compared to control (Table 8.1 and Fig. 8.3 A). The ethylene peak concentration was suppressed up to 1.7 fold in the fruit fumigated with BC (0.08 nmol kg^{-1} h^{-1}) and NC (0.06 nmol kg^{-1} h^{-1}), whilst the ethylene concentration of the fruit sprayed with BC and NC were not significantly different from untreated ones after 35 d cold storage (Table

8.1 and Fig. 8.3 A). After 50 d cold storage, the ethylene antagonists irrespective of the treatments significantly suppressed the concentration (up to 1.6 fold) but did not significantly delay the peak onset of 'Angeleno' plum as compared to control (Table 8.1 and Fig. 8.3 B). The significantly lowest ethylene peak concentration was exhibited in the fruit fumigated with NC and BC (0.07 and 0.08 nmol kg^{-1} h^{-1}). The MAP significantly delayed the ethylene peak onset and suppressed the peak concentration of 'Angeleno' plum as compared to the fruit without MAP after 35 d and 50 d cold storage periods (Table 8.1 and Fig. 8.3 A and B). The interaction effect between the treatments and the storage type on the ethylene peak onset and the concentration of 'Angeleno' plum was not significant after 35 d and 50 d cold storage periods (Table 8.1).

Ethylene production of 'Flavor Fall' pluot was in the range of 0.08-0.1 nmol kg^{-1} h^{-1} (Fig. 8.4). The ethylene peak onsets of 'Flavor Fall' pluot fumigated with ethylene antagonists (BC, NC and 1-MCP) were significantly delayed as compared to the control fruit after 40 d cold storage, whilst no significant effect of ethylene antagonists was observed after 60 d cold storage (Table 8.2 and Fig. 8.4 A). The ethylene antagonists, irrespective of the treatments, significantly suppressed (up to 1.3 fold) the ethylene peak concentration of Flavour Fall' pluot as compared to control after 40 d and 60 d cold storage periods. The ethylene peak concentrations of the fruit fumigated with BC (0.086 nmol kg^{-1} h^{-1}), NC (0.083 nmol kg^{-1} h^{-1}) and 1-MCP (0.083 nmol kg^{-1} h^{-1}) were significantly lowest, followed by the BC and NC spray solutions after 40 d cold storage. The ethylene peak concentration of the fruit fumigated with NC (0.078 nmol kg^{-1} h^{-1}), 1-MCP (0.080 nmol kg^{-1} h^{-1}) and BC (0.082 nmol kg^{-1} h^{-1}) were significantly lower as compared to the NC (0.091 nmol kg^{-1} h^{-1}) and BC (0.094 nmol kg^{-1} h^{-1}) spray solutions after 60 d cold storage. The MAP significantly delayed and suppressed the ethylene peak onset and the peak concentration of Flavour Fall' pluot as compared to the pluot without MAP in both cold storage periods (Table 8.2). There was a significant interaction effect between the treatments and the storage type on the ethylene peak onset of 'Flavor Fall' pluot after 40 d cold storage (Table 8.2). The ethylene peak onsets of the fruit treated with 1-MCP and BC fumigations in combination with MAP were significantly delayed (3 and 2 d, respectively) as compared to the fruit treated with 1-MCP and BC fumigations without MAP.

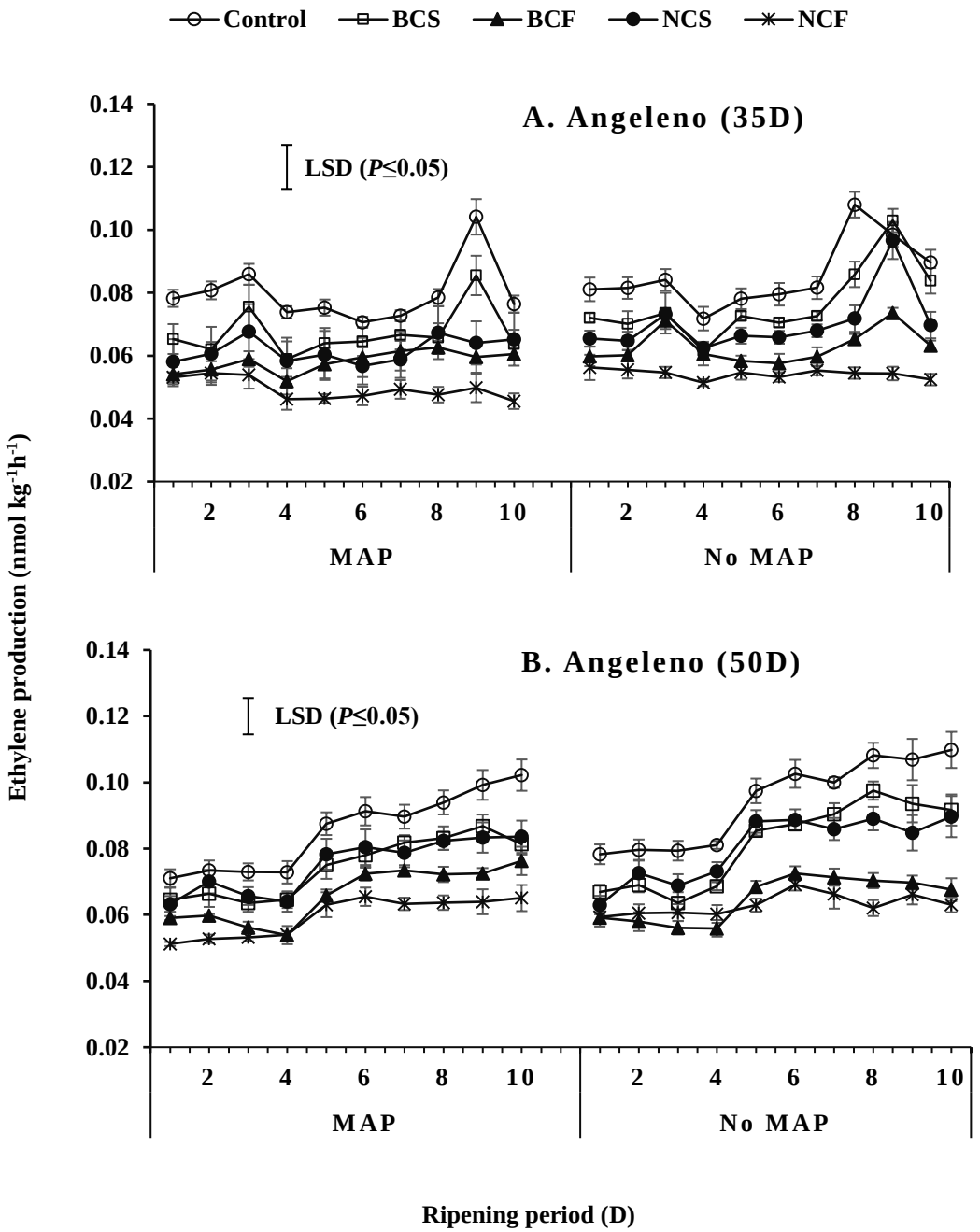

Fig. 8. 3 Effect of ethylene antagonists spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on ethylene production of 'Angeleno' plum stored at 1°C for 35 d (A) and 50 d (B). LSD bars represent treatments (tr) x days after storage (d). Vertical bars describe SE of means of three replicates and are not visible when the values are small. LSD values for ethylene production of 'Angeleno' plum (35 D): treatments (tr) =0.04, days after storage (d) =0.04 and their interaction (tr x d) =ns, 'Angeleno' plum (50 D): tr=0.03, d =0.034, tr x d =ns.

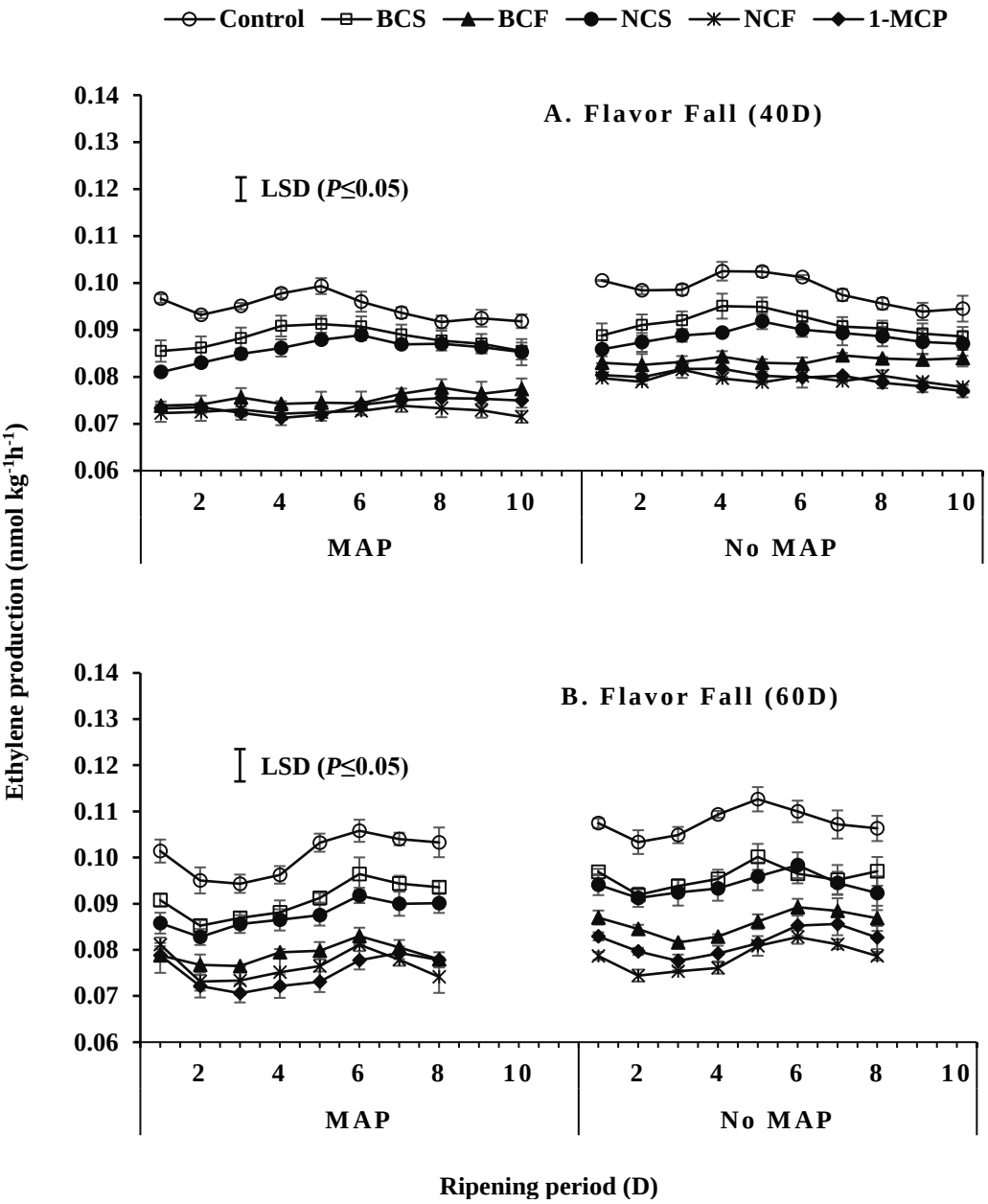

Fig. 8. 4 Effect of ethylene antagonists spray solutions (BCS and NCS) and fumigations (BCF, NCF and 1-MCP) on ethylene production of 'Flavor Fall' pluot stored for 40 and 60 d with or without MAP at 1°C. LSD bars represent treatments (tr) x days after storage (d). Vertical bars describe SE of means of three replicates and are not visible when the values are small. LSD values for ethylene production of 'Flavor Fall' pluot (40 D): treatments (tr) =0.002, days after storage (d) =0.001 and their interaction (tr x d) =ns, 'Flavor Fall' pluot (60 D): tr=0.003, d =0.002, tr x d =ns.

Table 8. 1 Effect of ethylene antagonists treated as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on the onset of ethylene peak (d) and the ethylene concentration (µmol $kg^{-1}h^{-1}$) of 'Angeleno' plum stored at cold storage (1 °C) for 35 and 50 d.

		Storage period (d)					
		35 d			50 d		
Parameters	Treatment	MAP	No MAP	Mean (Tr)	MAP	No MAP	Mean (Tr)
ethylene peak onset (d)	Control	8.0±0.0	7.3±0.3	7.7A	8.67±1.1	6.67±0.5	7.7
	BCS	9.0±0.0	8.0±0.0	8.5B	8.67±0.3	7.33±0.5	8.0
	BCF	9.7±0.3	8.3±0.3	9.0BC	8.00±0.5	7.00±0.5	7.5
	NCS	9.0±0.0	8.3±0.3	8.7B	8.33±0.3	7.33±0.5	7.8
	NCF	9.7±0.3	9.0±0.0	9.3C	9.00±0.8	8.33±0.5	8.7
	Mean (St)	9.07B	8.2A		8.53B	7.33A	
	LSD ($P \leq 0.05$)	Tr=0.48	st=0.31	Tr.st=ns	Tr=ns	st=1.03	Tr.st=ns
ethylene peak concentration (nmol $kg^{-1}h^{-1}$)	Control	0.09±0.01	0.11±0.0	0.10C	0.10±0.004	0.11±0.01	0.11 C
	BCS	0.09±0.01	0.10±0.0	0.09C	0.09±0.003	0.10±0.003	0.09 B
	BCF	0.08±0.01	0.07±0.0	0.08AB	0.08±0.003	0.07±0.003	0.08 A
	NCS	0.08±0.0	0.10±0.0	0.09BC	0.08±0.004	0.09±0.003	0.09 B
	NCF	0.06±0.0	0.07±0.0	0.06A	0.07±0.002	0.07±0.003	0.07 A
	Mean (St)	0.079A	0.089B		0.084	0.088	
	LSD ($P \leq 0.05$)	Tr=0.016	st=0.01	Tr.st=ns	Tr=0.01	st=ns	Tr.st=ns

n = 3 replicates (3 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP). Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 35 d and 50 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns.

Table 8. 2 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF, NCF and 1-MCP) with or without MAP on the onset of ethylene peak (d) and the ethylene concentration (µmol $kg^{-1}h^{-1}$) of 'Flavor Fall' pluot stored at cold storage (1 °C) for 40 and 60 d.

		Storage period (d)					
		40 d			60 d		
Parameters	Treatment	MAP	No MAP	Mean (Tr)	MAP	No MAP	Mean (Tr)
ethylene peak onset (d)	Control	4.7±0.3ab	4.3±0.3a	4.5A	7.3±0.54	5.3±0.72	6.3
	BCS	4.3±0.3a	4.3±0.3a	4.3A	6.7±0.54	5.0±0.00	5.8
	BCF	7.0±0.5de	5.0±0.5ab	6.0BC	6.3±0.27	6.0±0.00	6.2
	NCS	5.3±0.5abc	5.0±0.0ab	5.2AB	6.7±0.54	6.0±0.00	6.3
	NCF	6.7±0.7cde	6.0±0.0bcd	6.3C	5.7±0.27	6.3±0.27	6.0
	1-MCP	8.0±0.5e	5.0±0.5ab	6.5C	7.0±0.00	6.7±0.27	6.8
	Mean (St)	6.0B	4.94A		6.61B	5.89A	
	LSD ($P \leq 0.05$)	Tr=1.01	st=0.58	Tr.st=1.4	Tr=ns	st=0.57	Tr.st=ns
ethylene peak concentration (nmol $kg^{-1}h^{-1}$)	Control	0.11±0.001	0.12±0.002	0.11C	0.10±0.002	0.10±0.002	0.10D
	BCS	0.10±0.002	0.10±0.002	0.10B	0.09±0.003	0.10±0.003	0.09C
	BCF	0.08±0.002	0.09±0.001	0.09A	0.08±0.001	0.09±0.002	0.08B
	NCS	0.09±0.001	0.10±0.002	0.10B	0.09±0.002	0.09±0.003	0.09C
	NCF	0.08±0.001	0.08±0.001	0.08A	0.08±0.000	0.08±0.001	0.08A
	1-MCP	0.08±0.001	0.09±0.001	0.08A	0.08±0.001	0.08±0.001	0.08AB
	Mean (St)	0.091A	0.095B		0.085A	0.09B	
	LSD ($P \leq 0.05$)	Tr=0.005**	st=0.003*	Tr.st=ns	Tr=0.004	st=0.002	Tr.st=ns

n = 3 replicates (3 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP). Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 35 d and 50 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns.

8.3.2 Respiration rate

The respiration peak rates of 'Angeleno' plum were in the range of 0.81-1.1 mmol kg^{-1} h^{-1} (Table 8.3). The respiration peak onset and the respiration rate of 'Angeleno' plum treated with the ethylene antagonists (BC and NC) were significantly the same as compared to the untreated fruit in both cold storage periods, regardless of MAP (Table 8.3). There was no interaction effect between the treatments and the storage type on the respiration rate and the peak onset of 'Angeleno' plum in both cold storage periods (Table 8.3).

The respiration peak rates of 'Flavor Fall' pluot were in the range of 0.6-1.4 mmol kg^{-1} h^{-1} (Table 8.4). The respiration peak onset and the respiration peak rate of 'Flavor Fall' pluot treated with ethylene antagonists were not significantly different from the control after 40 d and 60 d cold storages. Flavour Fall' pluot stored with MAP had a significantly earlier respiration peak onset than control, however, the respiration rate was not significantly different as compared to the pluot without MAP in both cold storage periods (Table 8.4). The interaction between the treatments and the storage type was not significant on the respiration peak onset and the peak rate of 'Flavor Fall' pluot after 40 d and 60 d cold storages (Table 8.4).

Table 8. 3 Effect of ethylene antagonists treated as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on the onset of respiration peak (d) and the respiration peak rate (mmol $kg^{-1}h^{-1}$) of 'Angeleno' plum stored at cold storage (1 °C) for 35 and 50 d.

		Storage period (d)					
		35 d			50 d		
Parameters	Treatment	MAP	No MAP	Mean (Tr)	MAP	No MAP	Mean (Tr)
Respiration peak onset	Control	4.0±0.5	4.0±0.0	4.0	3.7±0.04	4.0±0.1	3.8
	BCS	4.3±0.0	4.3±0.7	4.3	4.0±0.03	4.0±0.1	4.0
	BCF	4.0±0.3	5.3±0.3	4.7	4.3±0.04	4.0±0.03	4.2
	NCS	5.0±0.0	4.0±0.0	4.5	3.7±0.1	4.0±0.04	3.8
	NCF	4.3±0.3	5.0±0.8	4.7	4.0±0.02	4.0±0.02	4.0
	Mean (St)	4.33	4.53		3.9	4.0	
	LSD ($P \leq 0.05$)	Tr=ns	st=ns	Tr.st=ns	Tr=ns	st=ns	Tr.st=ns
Respiration peak rate	Control	1.01±0.3	0.99±0.0	1.001	0.88±0.04	0.91±0.1	0.899
	BCS	1.05±0.3	0.94±0.0	0.995	0.91±0.01	0.82±0.04	0.864
	BCF	0.92±0.0	0.92±0.0	0.921	0.76±0.03	0.83±0.02	0.791
	NCS	1.06±0.5	0.95±0.0	1.007	0.90±0.06	0.82±0.03	0.859
	NCF	0.91±0.3	0.93±0.5	0.918	0.81±0.11	0.81±0.1	0.808
	Mean (St)	0.989	0.948		0.852	0.836	
	LSD ($P \leq 0.05$)	Tr=ns	st=ns	Tr.st=ns	Tr= ns	st=ns	Tr.st=ns

n = 3 replicates (3 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP). Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 35 d and 50 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns.

Table 8. 4 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF, NCF and 1-MCP) with or without MAP on the onset of respiration peak (d) and the respiration peak rate (mmol $kg^{-1}h^{-1}$) of 'Flavor Fall' pluot stored at cold storage (1 °C) for 40 and 60 d.

		Storage period (d)					
		40 d			60 d		
Parameters	Treatment	MAP	No MAP	Mean (Tr)	MAP	No MAP	Mean (Tr)
Respiration climacteric peak onset	Control	4.3±0.27	5.7±0.54	5.0	3.0±0.06	4.0±0.01	3.5
	BCS	4.3±0.27	4.7±0.27	4.5	4.7±0.10	4.3±0.04	4.5
	BCF	4.3±0.27	6.7±0.72	5.5	4.0±0.07	5.7±0.11	4.8
	NCS	4.7±0.27	6.7±0.72	5.7	4.3±0.05	5.7±0.07	5.0
	NCF	4.7±0.27	7.7±0.27	6.2	4.0±0.01	5.7±0.05	4.8
	1-MCP	6.7±0.72	5.7±0.54	6.2	4.3±0.04	5.3±0.04	4.8
	Mean (St)	4.83A	6.17B		4.06A	5.11B	
	LSD ($P \leq 0.05$)	Tr=ns	st=0.72	Tr.st=ns	Tr=ns	st=0.86	Tr.st=ns
Respiration climacteric peak rate	Control	0.94±0.47	0.83±0.00	0.89	1.27±0.15	1.13±0.02	1.20
	BCS	0.86±0.54	0.68±0.27	0.77	1.10±0.04	1.31±0.23	1.21
	BCF	0.96±0.00	0.91±0.72	0.94	1.27±0.06	1.13±0.02	1.20
	NCS	0.69±0.27	1.13±0.98	0.91	1.13±0.06	1.44±0.14	1.28
	NCF	0.93±0.00	0.93±0.72	0.93	1.17±0.01	1.27±0.05	1.22
	1-MCP	0.91±0.27	0.77±1.09	0.84	1.27±0.24	1.30±0.17	1.29
	Mean (St)	0.88	0.87		1.20	1.26	
	LSD ($P \leq 0.05$)	Tr=ns	st=ns	Tr.st=ns	Tr=ns	st=ns	Tr.st=ns

n = 3 replicates (3 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP). Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 35 d and 50 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns.

8.3.3 Physiological weight loss

The physiological weight losses of ‘Angeleno’ plum treated with ethylene antagonists were significantly lowered, irrespective of the treatments, as compared to the control (3.0 %) after 50 d cold storage (Table 8.5). The weight losses of the fruit treated with ethylene antagonist were not significantly different from the untreated fruit after 35 d cold storage (Table 8.5). The weight loss of ‘Angeleno’ plum stored with MAP (0.5 % and 0.72%, respectively on average) were significantly lower as compared to the plum without MAP (2.85 % and 4.32 %, respectively on average) after 35 d and 50 d cold storage (Table 8.5).

The physiological weight loss of ‘Flavor Fall’ pluot was not significantly affected by the ethylene antagonists after 40 d and 60 d cold storage (Table 8.6). However, the mean weight loss of the fruit fumigated with BC, NC and 1-MCP were comparatively up to 1.5 fold lower than the control fruit in both cold storage periods. The fruit stored with MAP (0.5 % and 0.86 %, respectively on average) exhibited significantly lower physiological weight loss as compared to the fruit without MAP (2.3 % and 3.19 %, respectively on average) after 40 d and 60 d cold storage. There was no significant interaction effect between the treatments and the storage type on the physiological weight loss of ‘Angeleno’ plum and ‘Flavor Fall’ pluot regardless of the cold storage periods.

8.3.4 Firmness

The firmness of ‘Angeleno’ plum treated with ethylene antagonists BC and NC were maintained significantly higher up to 1.2 fold as compared to untreated plum after 35 d cold storage (Table 8.5). The fruit fumigated with BC and NC maintained comparatively higher fruit firmness (1.1 fold each) as compared to the fruit treated with BC and NC spray solutions. After 50 d cold storage, firmness of the fruit treated with ethylene antagonists were not significantly different from the control fruit. However, mean firmness of the fruit treated with ethylene antagonists were tendentially higher ≈1.1 fold as compared to the untreated fruit. Firmness of the fruit stored with MAP were maintained significantly the same as compared to the fruit stored without MAP in both cold storage periods (Table 8.5). There was a significant interaction effect between the treatments and the storage type on the firmness of

‘Angeleno’ plum after 35 d cold storage. The fruit treated with BC spray solution in combination with MAP exhibited significantly higher firmness (1.1 fold) than the fruit treated with BC solution without MAP (Table 8.5).

The significant interaction effect was observed between the treatments and the storage type on the firmness of ‘Flavor Fall’ pluot after 40 d cold storage (Table 8.6). The fruit treated with BC and NC solutions in combination with MAP showed significantly higher firmness as compared to the ones without MAP after 40 d cold storage (Table 8.6). The firmness of Flavour Fall’ pluot treated with ethylene antagonists, except BC spray solution, were significantly higher up to 1.2 fold as compared to the untreated pluot after 40 d cold storage (Table 8.6). The fruit fumigated with BC, NC and 1-MCP were significantly firmer (1.1 fold each) than the control fruit and the fruit treated with BC and NC spray solutions after 60 d cold storage (Table 8.6). The fruit stored with MAP maintained significantly higher firmness as compared to the fruit without MAP in both cold storage periods (Table 8.6).

Table 8. 5 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on weight loss (%) and firmness (N) of 'Angeleno' plum stored at 1°C for 35 d and 50 d.

Treatments	Storage period (d)					
	35 d			50 d		
	MAP	No MAP	Mean(Tr.)	MAP	No MAP	Mean (Tr.)
	Weight Loss (%)					
Control	0.85±0.2	3.01±0.1	1.93	0.97±0.16a	5.03±0.09d	3.00B
BCS	0.21±0.2	2.69±0.1	1.45	0.60±0.01a	3.82±0.07b	2.21A
BCF	0.63±0.01	2.91±0.04	1.77	0.79±0.13a	3.79±0.22b	2.29A
NCS	0.39±0.2	2.79±0.2	1.59	0.61±0.02a	4.49±0.27c	2.55A
NCF	0.41±0.2	2.87±0.2	1.64	0.63±0.02a	4.44±0.21c	2.53A
Mean(st)	0.50A	2.85B		0.72A	4.32B	
LSD ($P \leq 0.05$)	Tr=ns	st=0.20**	Tr.st =ns	Tr=0.32* *	st=0.20**	Tr.st =ns
	Firmness (N)					
Control	36.7±0.5bc	33.5±0.5a	35.1A	34.8±0.51	31.0±1.04	32.90
BCS	40.0±0.6d	35.4±3.7ab	37.7B	35.4±3.23	35.6±0.54	35.46
BCF	40.0±0.3d	39.2±0.5cd	39.6BC	36.3±0.10	36.5±0.42	36.39
NCS	39.1±1.0cd	38.8±0.9cd	38.9BC	35.3±0.41	35.2±0.32	35.23
NCF	41.4±0.8 d	40.5±0.6d	40.9C	37.4±0.67	35.7±0.83	36.57
Mean(st)	39.44	37.47		35.83	34.78	
LSD ($P \leq 0.05$)	Tr=2.6	st=ns	Tr.st=2.3	Tr=ns	st=ns	Tr.st=ns

n = 3 replicates (12 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP), ns=non-significance. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 35 d and 50 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns.

Table 8. 6 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on the weight loss (%) and firmness (N) of 'Flavor Fall' pluot stored at 1°C for 40 d and 60 d.

Treatments	Storage period (d)					
	40 d			60 d		
	MAP	No MAP	Mean (Tr.)	MAP	No MAP	Mean (Tr.)
	Weight Loss (%)					
Control	0.91±0.1	2.52±0.3	1.72	1.03±0.3	3.40±0.2	2.21
BCE	0.54±0.0	2.40±0.2	1.47	0.89±0.1	3.32±0.1	2.11
BCF	0.48±0.0	2.07±0.1	1.28	0.78±0.1	2.99±0.1	1.88
NCE	0.33±0.1	2.47±0.2	1.40	0.77±0.1	3.12±0.1	1.94
NCF	0.33±0.1	2.02±0.3	1.17	1.05±0.1	3.17±0.3	2.11
1-MCP	0.39±0.2	2.36±0.2	1.38	0.66±0.1	3.18±0.1	1.92
Mean (st.)	0.50A	2.30 B		0.86 A	3.19 B	
LSD (P≤ 0.05)	Tr=ns	st=0.3**	Tr.st=ns	Tr=ns	st=0.3 **	Tr.st= ns
	Firmness (N)					
Control	32.1±2.0ab	29.6±0.9a	30.8 A	33.6±0.7	26.3±0.4	30.0A
BCE	34.4±0.5bcd	30.3±1.4a	32.3 A	30.8±0.8	29.3±0.7	30.0A
BCF	36.2±0.8cd	35.0±0.4cd	35.6 B	35.0±1.1	31.4±0.5	33.2B
NCE	36.6±0.5d	33.5±0.3bc	35.1 B	34.5±1.0	30.0±0.5	32.2AB
NCF	36.7±0.7d	35.2±0.5cd	35.9 B	34.1±0.3	31.4±0.5	32.7B
1-MCP	36.8±0.7d	36.0±0.7cd	36.4 B	35.9±0.7	30.0±0.8	32.9B
Mean (st.)	35.0 B	33.7 A		34.0 B	29.72 A	
LSD (P≤ 0.05)	Tr=1.9**	st=1.1*	Tr.st=2.7*	Tr=2.2*	st=1.3**	Tr.st=ns

n = 3 replicates (12 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP), ns=non-significance. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 40 d and 60 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns.

8.3.5 SSC, TA and SSC:TA

There were significant interaction effects between treatments and storage type on SSC content of 'Angeleno' plum after 35 d and 50 d cold storage. The ethylene antagonist (NC), either as fumigation or as spray solution, in combination with MAP, was observed to be the most effective in maintaining lower SSC content of 'Angeleno' plum in both storage periods (Table 8.7). The soluble solids content of 'Angeleno' plums treated with ethylene antagonists, except BC and NC spray solutions in 50 d cold storage, were significantly lowered up to 2.6 % and 3.6 % as compared to control after 35 d and 50 d cold storage, respectively (Table 8.7). The fruit stored with MAP maintained significantly lower SSC content (1.1 fold each) than the fruit without MAP after 35 d and 50 d cold storage (Table 8.7). There were interaction effects between the treatments and the storage type on TA and SSC:TA ratio of 'Angeleno' plum in both cold storage periods (Table 8.7). 'Angeleno' plum treated with BC or NC fumigations maintained significantly higher TA content (up to 1.1 fold) and lower SSC:TA ratio (up to 1.2 fold) as compared to control after 50 d cold storage, whilst no significant treatment effect was observed after 35 d cold storage (Table 8.7). The fruit stored in MAP retained significantly higher TA contents and lower SSC:TA ratio as compared to the fruit without MAP after 35 d and 50 d cold storage.

The interaction effects between treatments and storage type on SSC content of 'Flavor Fall' pluot were significant in both 40 d and 60 d cold storage periods. 'Flavor Fall' pluot treated with NC fumigation and stored in MAP bags exhibited significantly lowest SSC content in both cold storage periods (Table 8.8). When averaged, the fruit stored with MAP retained significantly lower SSC content (4.1 and 1.3 %, respectively) than the fruit without MAP after 40 d and 60 d cold storage. The significant interaction effect between treatments and storage type on TA content and SSC:TA ratio of 'Flavor Fall' pluot was recorded in 60 d cold storage. The fruit treated with ethylene antagonists maintained significantly higher TA content (up to 1.1 and 1.2 fold, respectively) and lower SSC:TA ratio (up to 1.1 and 1.2 fold, respectively) as compared to the untreated fruit after 40 d and 60 d cold storage irrespective of the treatments. The fruit stored with MAP significantly increased TA content (1.1 and 1.2 fold, respectively) than the fruit without MAP after 40 d and 60 d cold storage. SSC:TA ratio was significantly lowered (1.2 fold) in the fruit stored with MAP than

the fruit without MAP after 60 d cold storage, while no significant difference was observed after 40 d cold storage.

Table 8. 7 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on the SSC (%), TA (%) and SSC:TA ratio of 'Angeleno' plum stored at 1°C for 35 d and 50 d.

Treatments	Storage period (d)					
	35 d			50 d		
	MAP	No MAP	Mean (Tr.)	MAP	No MAP	Mean (Tr.)
			SSC (%)			
Control	15.10±0.03c	16.07±0.03e	15.67D	15.27±0.00bc	15.70±0.00d	15.40C
BCS	15.20± 0.03c	15.73±0.03d	15.50C	15.27±0.16c	15.57±0.05d	15.38C
BCF	15.00±0.00 b	15.73±0.05d	15.37B	14.93±0.05b	15.13±0.03bc	15.03AB
NCS	14.47±0.05ab	15.63±0.03d	15.27A	14.90±0.05a	15.63±0.03d	15.05B
NCF	14.50±0.032a	15.70±0.00d	15.27A	14.83±0.00a	15.23±0.05c	14.87A
Mean (st)	15.05A	15.77B		14.8A	15.5B	
LSD (P≤ 0.05)	tr=0.08**	st=0.05**	tr.st =0.12**	tr=0.17**	st=0.11**	tr.st =0.24**
			TA (%)			
Control	1.47±0.00	1.36±0.02	1.42	1.27±8.0.03a	1.36±0.07abcd	1.32A
BCS	1.50±0.02	1.34±0.00	1.42	1.50±0.05bde	1.34±0.00ab	1.42ABC
BCF	1.45±0.02	1.41±0.03	1.43	1.29±0.036a	1.52±0.07ef	1.43BC
NCS	1.50±0.02	1.34±0.00	1.42	1.34±0.00ab	1.39±0.04abcde	1.34AB
NCF	1.56±0.05	1.39±0.04	1.47	1.34±0.03abc	1.65±0.02f	1.50C
Mean (st)	1.50B	1.37A		1.35A	1.45B	
LSD (P≤ 0.05)	tr=ns	st=0.03**	tr.st=ns	tr=0.1*	st=0.06*	tr.st =0.14*
			SSC:TA			
Control	10.52±0.15	11.44±0.24	10.98	11.97±0.42c	11.52±0.62c	11.74C
BCS	9.92±0.13	11.72±0.00	10.82	10.12±0.32ab	11.72±0.00c	10.9ABC
BCF	10.18±0.00	11.55±0.14	10.87	11.55±0.29c	10.09±0.45ab	10.46AB
NCS	9.96±0.09	11.67±0.02	10.81	10.82±0.00bc	10.95±0.26bc	11.25BC
NCF	9.51±0.29	11.39±0.30	10.59	10.81±0.22bc	9.46±0.10a	10.14A
Mean (st)	10.07A	11.55B		11.06	10.75	
LSD (P≤ 0.05)	tr=ns	st=0.24**	Tr.st=ns	Tr=0.85*	st=ns	tr.st =1.2*

n = 3 replicates (12 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP), ns=non-significance. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 35 d and 50 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 8. 8 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on SSC (%), TA (%) and SSC:TA ratio of 'Flavor Fall' pluot stored at 1°C for 40 d and 60 d.

Treatments	Storage period (d)					
	40 d			60 d		
	MAP	No MAP	Mean (Tr.)	MAP	No MAP	Mean(Tr.)
			SSC (%)			
Control	15.1±0.0c	15.7±0.0e	15.4D	15.1±0.0de	15.4±0.1f	15.3B
BCS	14.7±0.0b	15.3±0.0d	15.0B	14.6±0.1a	15.5±0.0f	15.1A
BCF	15.1±0.1c	15.3±0.0d	15.2C	14.9±0.0bc	15.2±0.0de	15.0A
NCS	14.3±0.0a	15.3±0.0d	14.8A	14.8±0.0ab	15.1±0.0d	14.9A
NCF	14.8±0.0b	15.5±0.0e	15.2C	14.6±0.0a	15.3±0.0ef	15.0A
1-MCP	15.7±0.0c	15.7±0.0d	15.2C	15.0±0.0bcd	15.0±0.1cd	15.0A
Mean (st)	14.8A	15.4B		14.9A	15.1B	
LSD ($P \leq 0.05$)	Tr=0.12**	st=0.07**	Tr.st =0.18**	Tr=0.2**	st=0.1**	Tr.st =0.2**
			TA (%)			
Control	2.03±0.0	1.74±0.0	1.89A	1.7±0.0cd	1.5±0.0a	1.56A
BCS	2.03±0.0	2.03±0.0	2.03B	1.8±0.0e	1.5±0.0ab	1.68B
BCF	2.06±0.0	2.03±0.1	2.04B	1.9±0.0f	1.7±0.0cd	1.82C
NCS	2.10±0.0	1.94±0.0	2.02B	1.8±0.0de	1.7±0.0cd	1.73B
NCF	2.10±0.1	2.01±0.1	2.06B	2.0±0.0fg	1.6±0.0bc	1.81C
1-MCP	2.01±0.0	2.01±0.0	2.01B	2.1±0.1g	1.7±0.0cd	1.89C
Mean (st)	2.05B	1.96A		1.9B	1.6A	
LSD ($P \leq 0.05$)	Tr=0.11*	st=0.06*	Tr.st=ns	Tr=0.1**	st=0.04**	Tr.st =0.1**
			SSC:TA			
Control	7.27±0.1	7.65±0.1	7.46A	8.69±0.1cde	10.7±0.5g	9.72C
BCS	8.44±0.0	7.55±0.1	7.99B	8.18±0.1bc	9.90±0.1f	9.03B
BCF	7.43±0.2	7.45±0.1	7.44A	7.55±0.0ab	9.21±0.1e	8.29A
NCS	7.34±0.0	7.29±0.1	7.32A	8.37±0.1cd	8.89±0.1de	8.64AB
NCF	7.52±0.2	7.51±0.3	7.52A	7.55±0.1ab	9.21±0.1e	8.38A
1-MCP	7.50±0.0	7.60±0.0	7.55A	7.23±0.2a	9.21±0.0e	8.22A
Mean (st)	7.58	7.51		7.9A	9.5B	
LSD ($P \leq 0.05$)	Tr=0.4*	st=ns	Tr.st=ns	Tr=0.4**	st=0.3**	Tr.st =0.6*

n = 3 replicates (12 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP), ns=non-significance. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 40 d and 60 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

8.3.6 Individual sugars

Glucose, fructose, sucrose and sorbitol were identified in the experimental fruit. The concentrations of individual sugars and sugar-alcohol increased with the extension of the cold storage period in 'Angeleno' plum (Table 8.9) whilst they decreased with longer cold storage period in 'Flavor Fall' pluot (Table 8.10). There were significant interaction effects between treatments and storage type on glucose level of 'Angeleno' plum after 35 d cold storage and on sucrose levels of 'Angeleno' plum after 35 d and 50 d cold storage. BC and NC treatments, except BC spray solution, maintained significantly lower glucose (≈ 1.1 fold) and significantly higher sucrose (1.4 and 1.5 fold, respectively) in 'Angeleno' plum treated with MAP as compared to the fruit without MAP after 35 d and 50 d cold storage. Fructose concentrations of 'Angeleno' plums fumigated with BC and NC were maintained significantly lower as compared to control (203.5 g kg^{-1}) after 35 d cold storage (Table 8.9) while they were not significantly different to control after 50 d cold storage. 'Angeleno' plum stored with MAP maintained significantly lower glucose, fructose and sorbitol (≈ 1.1 fold each) and significantly higher sucrose (1.4 and 1.5 fold, respectively) as compared to the fruit without MAP after 35 d and 50 d cold storage. The levels of sorbitol in 'Angeleno' plums treated with ethylene antagonists were lower, although the concentrations were not significantly different, than the untreated plum after 35 d and 50 d cold storage.

Glucose and fructose concentrations of 'Flavor Fall' pluot treated with ethylene antagonists (BC, NC and 1-MCP) were not significantly different as compared to control after 40 d and 60 d cold storages (Table 8.10). The significant interaction effect between treatments and storage type was observed on the sucrose level of the pluot stored for 40 and 60 days. Sucrose concentrations of MAP-stored pluot which are treated with BC spray solutions (28.65 and 17.72 g kg^{-1}, respectively) and BC fumigation (28.65 and 19.93 g kg^{-1}, respectively) were significantly higher as compared to control (20.23 and 12.61 g kg^{-1}, respectively) after 40 d and 60 d cold storage periods. MAP-stored pluot fumigated with NC fumigation showed significantly highest sucrose concentration (24. 62 g kg^{-1}) after 60 d cold storage compared to control and rest of the fruit. The sorbitol concentration of 'Flavor Fall' pluot fumigated with BC and NC, regardless of MAP, were significantly higher when compared to control (121.2 g kg^{-1}) after 60 days cold storage. 1-MCP fumigated fruit

stored without MAP showed the highest concentration of sorbitol (153.9 g kg^{-1}) after 60 days cold storage.

Table 8. 9 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on the levels of individual sugars of 'Angeleno' plum stored at 1°C for 35 d and 50 d.

Treatments	Storage period (d)					
	35 d			50 d		
	MAP	No MAP	Mean(Tr.)	MAP	No MAP	Mean(Tr.)
	Glucose (g kg^{-1})					
Control	183.7±2.42a	222.8±1.96d	203.3b	211.8±5.25	219.3±5.47	215.5
BCS	199.0±1.29b	211.2±2.28cd	205.1b	207.8±13.69	241.5±5.79	224.6
BCF	182.3±4.48a	200.5±2.48bc	191.4a	209.8±9.52	226.4±1.42	218.1
NCS	191.8±5.33ab	220.9±3.33d	206.3b	207.5±5.43	233.6±7.72	220.6
NCF	185.0±2.60a	197.1±5.52b	191.0a	193.9±7.39	220.3±2.72	207.1
Mean(st)	188.3A	210.5B		206.2A	228.2B	
LSD (P≤ 0.05)	Tr=8.04**	st=5.04**	Tr.st =11.37*	Tr=ns	st=11.74**	Tr.st =ns
	Fructose (g kg^{-1})					
Control	176.3±2.619	203.5±2.845	189.9b	210.1±5.614	217.1±8.676	213.6
BCS	183.2±1.406	192.2±2.332	187.7b	198.3±14.518	231.9±8.819	215.1
BCF	167.0±5.239	181.4±2.146	174.2a	198.9±11.975	214.6±4.294	206.7
NCS	183.2±4.527	201.4±3.738	192.3b	199.0±5.042	219.4±8.699	209.2
NCF	170.8±4.308	183.3±1.613	177.0a	180.1±6.779	201.9±2.449	191.0
Mean(st)	176.08A	192.35B		197.3A	217.0B	
LSD (P≤ 0.05)	Tr=**	st=**	Tr.st =ns	Tr=ns	st =13.37*	Tr.st =ns
	Sucrose (g kg^{-1})					
Control	18.16±0.696b	8.97 ±0.174a	13.56a	22.35±0.58bc	13.44±0.27a	17.89a
BCS	17.79±0.890b	15.88±0.395b	16.84b	19.61±0.59b	19.43±0.63b	19.52a
BCF	35.57±1.388d	26.60±0.585c	31.08d	43.74±2.26d	27.13±0.26c	35.43b
NCS	15.79±0.662b	10.73±0.217a	13.26a	16.84±0.73ab	16.59±0.80ab	16.72a
NCF	32.44±1.929d	23.77±1.276c	28.10c	50.66±3.179e	25.64±1.82c	38.15b
Mean(st)	23.95B	17.19A		30.64B	20.45A	
LSD (P≤ 0.05)	Tr=2.59**	st=1.64**	Tr.st =3.67*	Tr=3.84**	st =2.43**	Tr.st =5.43**
	Sorbitol (g kg^{-1})					
Control	167.4±2.323	179.5±1.512	173.4	193.6±1.830	195.4±7.554	194.5
BCS	162.7±1.454	182.6±3.271	172.7	181.8±10.465	213.5±5.350	197.6
BCF	161.4±3.186	185.0±1.917	173.2	189.3±9.145	196.7±1.383	193.0
NCS	157.6±3.709	179.8±1.650	168.7	178.7±4.850	201.2±9.257	189.9
NCF	161.3±3.162	176.6±3.059	169.0	186.9±5.276	179.7±1.660	183.3
Mean(st)	162.1A	180.7B		186.1	197.3	
LSD (P≤ 0.05)	Tr=ns	st=4.31**	Tr.st =ns	Tr=ns	st =10.54*	Tr.st =ns

n = 3 replicates (12 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP), ns=non-significance. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 35 d and 50 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 8. 10 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on the levels of individual sugars of 'Flavor Fall' pluot stored at 1°C for 40 d and 60 d.

Treatments	40 d			60 d		
	MAP	No MAP	Mean (Tr.)	MAP	No MAP	Mean (Tr.)
			Glucose (g kg^{-1})			
Control	189.4±2.7cd	197.8±1.6def	193.6	170.2±5.5	156.2±5.1	163.2
BCS	178.4±1.5ab	203.4±4.4f	190.9	163.2±2.5	153.9±3.5	158.6
BCF	184.3±0.4abc	188.3±3.0bcd	186.3	158.2±0.4	164.8±2.4	161.5
NCS	176.6±4.4a	200.2±5.1ef	188.4	172.4±6.6	152.6±2.9	162.5
NCF	190.6±2.7cde	185.0±2.5abc	187.8	157.8±5.3	157.5±0.4	157.7
1-MCP	191.6±3.5cde	185.4±1.0abc	188.5	157.4±1.7	168.0±2.6	162.7
Mean (st)	185.14 A	193.33 B		163.2	158.8	
LSD (P≤ 0.05)	Tr =ns	st =3.88**	Tr.St =9.51**	Tr =ns	St =ns	Tr.St =ns
			Fructose (g kg^{-1})			
Control	186.3±1.6	193.5±2.1	189.9	173.5±7.2c	143.3±3.0a	158.4
BCS	187.1±2.9	204.2±6.3	195.6	160.7±2.9abc	145.8±3.2a	153.3
BCF	194.5±2.7	191.5±1.8	193.0	166.2±2.3bc	161.0±5.0abc	163.6
NCS	179.6±4.7	190.2±8.4	184.9	168.1±6.3bc	149.1±7.2ab	158.6
NCF	188.6±3.3	186.1±4.5	187.3	159.6±6.4abc	158.3±1.3abc	159.0
1-MCP	193.1±5.0	180.8±2.0	186.9	153.7±1.9ab	166.2±3.0bc	160.0
Mean (st)	188.2	191.0		163.6B	154.0A	
LSD (P≤ 0.05)	Tr =ns	st =ns	Tr.St =ns	Tr =ns	St =6.84*	Tr.St =16.8*
			Sucrose (g kg^{-1})			
Control	20.21±0.5a	20.26±1.8a	20.23A	11.71±0.8a	12.61±1.3ab	12.2A
BCS	28.65±1.7c	18.47±0.7a	23.56ABC	17.72±1.4cd	13.69±0.8abc	15.7BC
BCF	28.01±1.3c	19.22±0.8a	23.61ABC	19.93±0.2d	15.53±1.2abcd	17.7CD
NCS	26.25±1.7bc	18.54±1.7a	22.39AB	13.79±0.9abc	13.60±0.3abc	13.7AB
NCF	25.51±0.7bc	28.26±1.5c	26.89C	24.62±0.6e	13.76±0.4abc	19.2D
1-MCP	21.30±1.1ab	27.54±1.5c	24.42BC	19.61±2.0d	16.38±1.4bcd	18.0CD
Mean (st)	24.99 B	22.05 A		17.89 B	14.26 A	
LSD (P≤ 0.05)	Tr = 3.35*	st =1.94*	Tr.St =4.74**	Tr =2.82**	St =1.63**	Tr.St =3.98*
			Sorbitol (g kg^{-1})			
Control	157.3±1.4a	172.4±2.8b	164.8AB	144.2±4.3bcd	121.2±1.8a	132.7A
BCS	165.0±3.1ab	172.4±3.4b	168.7B	130.8±1.8ab	136.3±5.0bc	133.5A
BCF	159.2±2.1a	157.4±0.3a	158.3A	150.6±2.8cd	137.8±4.4bc	144.2B
NCS	161.5±2.7a	161.3±2.5a	161.4A	136.1±5.1bc	134.6±4.5ab	135.4AB
NCF	158.7±1.8a	166.3±2.4ab	162.5AB	149.8±3.6cd	138.6±1.7bc	144.2B
1-MCP	158.0±2.9a	159.2±3.5a	158.6A	133.4±0.6ab	153.9±5.0d	143.7B
Mean (st)	159.93 A	164.86 B		140.8	137.1	
LSD (P≤ 0.05)	Tr =6.13*	st =3.54*	Tr.St =8.66*	Tr =9.16*	St =ns	Tr.St =12.96**

n = 3 replicates (12 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP), ns=non-significance. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 40 d and 60 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns.

8.3.7 Individual organic acids

Malic, citric, fumaric and succinic acids were identified in the tested fruit. In all cases, the concentrations of individual organic acids were reduced, except citric acid which was increased, with longer cold storage periods in both 'Angeleno' plum (Table 8.11) and 'Flavor Fall' pluot (Table 8.12). There was a significant interaction effect between treatments and storage type on individual organic acids after 35 d cold storage. Malic acid concentrations of 'Angeleno' plum treated with ethylene antagonists, except BC spray solution, and MAP were maintained significantly higher up to 1.4 fold as compared to the untreated plum with or without MAP after 35 d cold storage (Table 8.11). Malic acid concentrations of 'Angeleno' plum treated with BC and MAP was observed to be the highest (26.48 g kg^{-1}), followed by NC spray without MAP (26.36 g kg^{-1}) and NC fumigation without MAP (26.23 g kg^{-1}) after 35 days storage. The fruit treated with NC spray solution and stored without MAP resulted in the highest succinic concentration (4.79 g kg^{-1}) after 35 days storage. Regardless of MAP, citric concentration in the fruit treated with NC and fumaric concentration in the fruit treated with BC and NC, except BC spray solution, remained significantly lower than control after 35 d cold storage. Citric and fumaric acids were not significantly affected by ethylene antagonists and MAP after 50 d cold storage.

Malic, citric, fumaric and succinic acids were identified in 'Flavor Fall' pluot. All the individual organic acids were reduced, except fumaric acid which was increased, with increasing cold storage periods in 'Flavor Fall' pluot (Table 8.12). When averaged, the malic acid concentration of 'Flavor Fall' pluot fumigated with BC, NC and 1-MCP were significantly higher ≈1.1 fold each as compared to control and spray solutions of BC and NC after 40 d cold storage (Table 8.12). Malic acid of 'Flavor Fall' pluot treated with BC, and NC spray solutions and 1-MCP in the presence of MAP was maintained significantly higher as compared to control without MAP, however, they were not significantly different from the control with MAP after 60 d cold storage. Citric acid concentrations of the fruit treated with BC and NC spray solutions and BC fumigation were maintained at significantly higher levels (≈1.1 fold) as compared to control and other treatments after 40 d cold storage. Fumaric acid concentrations of the fruit treated with BC and NC, except BC fumigation, in the presence of MAP were significantly higher (≈1.1 fold each) as compared to control after 40 d cold storage.

Succinic acid concentration of the fruit fumigated with 1-MCP (5.24 g kg^{-1}) in the presence of MAP was significantly higher as compared to control with and without MAP (3.99 and 4.54 g kg^{-1}, respectively) after 40 d cold storage. The levels of citric, fumaric and acids of the fruit treated with ethylene antagonists were not significantly different from the untreated fruit after 60 d cold storage.

Table 8. 11 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on the levels of organic acids in the pulp of 'Angeleno' plum fruit stored at 1°C for 35 d and 50 d.

Treatments	Storage period (d)					
	35 d			50 d		
	MAP	No MAP	Mean(Tr.)	MAP	No MAP	Mean(Tr.)
	Malic acid (g kg^{-1})					
Control	14.21±0.16b	14.98±0.02b	14.60A	3.624±0.16	3.512±0.11	3.568A
BCS	12.78±0.08a	15.16±0.26b	13.97A	3.582±0.01	4.292±0.14	3.937B
BCF	26.48±0.67f	16.46±0.11c	21.47B	3.825±0.13	4.322±0.03	4.073B
NCS	24.77±0.30d	26.36±0.42ef	25.57C	4.096±0.01	4.062±0.09	4.079B
NCF	25.38±0.44de	26.23±0.17ef	25.81C	3.708±0.10	3.997±0.27	3.853AB
Mean (st)	20.73B	19.84A		3.77A	4.04B	
LSD (P≤ 0.05)	tr=0.72**	st=0.45**	tr.st =1.01**	tr=0.31*	st=0.19*	tr.st=ns
	Citric acid (g kg^{-1})					
Control	0.88±0.003cd	0.85±0.014c	0.867C	0.090±0.000	0.111±0.027	0.100
BCS	0.85±0.003c	0.89±0.003cd	0.871C	0.089±0.002	0.092±0.002	0.091
BCF	0.49±0.020a	0.91±0.002d	0.701B	0.087±0.001	0.090±0.000	0.089
NCS	0.53±0.024ab	0.52±0.011ab	0.523A	0.092±0.002	0.090±0.000	0.091
NCF	0.49±0.025a	0.56±0.029b	0.529A	0.087±0.001	0.086±0.002	0.086
Mean (st)	0.65A	0.75B		0.09	0.09	
LSD (P≤ 0.05)	tr=0.04**	st=0.02**	tr.st =0.05**	tr=ns	st=ns	tr.st=ns
	Fumaric acid (g kg^{-1})					
Control	0.386±0.000g	0.378±0.000e	0.382E	0.077±0.000	0.104±0.026	0.091
BCS	0.381±0.000f	0.377±0.00de	0.379D	0.075±0.000	0.073±0.000	0.074
BCF	0.079±0.000c	0.376±0.000d	0.227C	0.075±0.001	0.075±0.000	0.075
NCS	0.079±0.000c	0.073±0.000b	0.076B	0.076±0.000	0.074±0.000	0.075
NCF	0.078±0.001c	0.071±0.000a	0.074A	0.077±0.000	0.077±0.001	0.077
Mean (st)	0.2A	0.25B		0.08	0.08	
LSD (P≤ 0.05)	tr=0.001**	st=0.01**	tr.st=0.002 **	tr=ns	st=ns	tr.st=ns
	Succinic acid (g kg^{-1})					
Control	3.99±0.016ab	3.84±0.047a	3.914A	0.967±0.017	0.862±0.020	0.914A
BCS	3.84±0.019a	3.81±0.069a	3.826A	1.009±0.008	1.047±0.036	1.028B
BCF	4.43±0.164c	4.06±0.049ab	4.247B	1.039±0.033	1.059±0.018	1.049B
NCS	4.49±0.077c	4.79±0.051d	4.639C	1.138±0.013	1.106±0.016	1.122C
NCF	4.13±0.053b	4.43±0.038c	4.277B	1.099±0.014	1.064±0.042	1.081BC
Mean (st)	4.18	4.19		1.05	1.03	
LSD (P≤ 0.05)	Tr=0.17**	st=ns	Tr.st =0.25*	Tr=0.053**	st =ns	Tr.st =ns

n = 3 replicates (12 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP), ns=non-significance. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 35 d and 50 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 8. 12 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on the levels of organic acids of 'Flavor Fall' pluot stored at 1°C for 40 d and 60 d.

Treatments	Storage period (d)					
	40 d			60 d		
	MAP	No MAP	Mean (Tr.)	MAP	No MAP	Mean (Tr.)
			Malic acid (g kg^{-1})			
Control	15.5±0.3	14.6±0.1	15.0A	2.6±0.0cde	2.5±0.1bc	2.53
BCS	16.0±0.1	14.3±0.3	15.1A	2.8±0.0de	2.1±0.1a	2.47
BCF	17.2±0.2	15.0±0.1	16.1B	2.6±0.0bcde	2.4±0.0bc	2.49
NCS	15.7±0.1	14.6±0.6	15.2A	2.8±0.0e	2.4±0.1bc	2.63
NCF	17.0±0.1	15.5±0.3	16.3B	2.3±0.1ab	2.6±0.1bcd	2.45
1-MCP	16.4±0.3	15.9±0.2	16.1B	2.8±0.0de	2.3±0.0abc	2.56
Mean(st)	16.3B	15.0A		2.66B	2.39A	
LSD ($P \leq 0.05$)	Tr=0.7**	st=0.4**	Tr.st =ns	Tr=ns	st=0.1**	Tr.st =0.2**
			Citric acid (g kg^{-1})			
Control	0.23±0.0	0.19±0.0	0.21A	0.17±0.0	0.16±0.0	0.16
BCS	0.25±0.0	0.23±0.0	0.24C	0.16±0.0	0.16±0.0	0.16
BCF	0.26±0.0	0.22±0.0	0.24C	0.17±0.0	0.16±0.0	0.17
NCS	0.24±0.0	0.22±0.0	0.23BC	0.17±0.0	0.17±0.0	0.17
NCF	0.21±0.0	0.22±0.0	0.22AB	0.16±0.0	0.17±0.0	0.17
1-MCP	0.22±0.0	0.22±0.0	0.22ABC	0.17±0.0	0.16±0.0	0.17
Mean(st)	0.23B	0.22A		0.17B	0.16A	
LSD ($P \leq 0.05$)	Tr=0.02*	st=0.01*	Tr.st=ns	Tr=ns	st=0.003*	Tr.st=ns
			Fumaric acid (g kg^{-1})			
Control	0.07±0.0a	0.08±0.0ab	0.07A	1.14±0.0c	0.89±0.0a	1.02
BCS	0.09±0.0de	0.07±0.0a	0.08BC	1.01±0.0b	1.00±0.0b	1.00
BCF	0.08±0.0ab	0.08±0.0abc	0.08AB	1.03±0.0b	0.99±0.0b	1.01
NCS	0.08±0.0cd	0.08±0.0bcd	0.08C	1.07±0.0bc	1.02±0.0b	1.04
NCF	0.09±0.0e	0.08±0.0abc	0.09C	1.04±0.0b	1.04±0.0b	1.04
1-MCP	0.08±0.0abc	0.08±0.0cd	0.08C	0.98±0.0b	0.99±0.0b	0.99
Mean(st)	0.08B	0.08A		1.05	0.99	
LSD ($P \leq 0.05$)	Tr =0.004**	st=0.003*	Tr.st =0.006**	Tr=ns	st=0.03*	Tr.st =0.08*
			Succinic acid (g kg^{-1})			
Control	4.54±0.1bcd	3.99±0.1a	4.37A	0.15±0.0	0.14±0.0	0.15
BCS	4.33±0.1abc	4.33±0.3abc	4.33A	0.14±0.0	0.15±0.0	0.14
BCF	4.71±0.2bcd	4.48±0.0abcd	4.60AB	0.15±0.0	0.14±0.0	0.15
NCS	4.91±0.1de	4.19±0.1ab	4.56AB	0.15±0.0	0.15±0.0	0.15
NCF	4.65±0.1bcd	4.81±0.1cde	4.73B	0.15±0.0	0.14±0.0	0.15
1-MCP	5.24±0.1e	4.34±0.2abc	4.79B	0.15±0.0	0.15±0.0	0.15
Mean(st)	4.73B	4.36A		0.15B	0.14A	
LSD ($P \leq 0.05$)	Tr=0.33*	st=0.2**	Tr.st=0.5*	Tr=ns	st=0.001*	Tr.st =ns

n = 3 replicates (12 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP), ns=non-significance. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 40 d and 60 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns.

8.3.8 Total phenols, ascorbic acid and antioxidant capacity

Total phenolic contents of 'Angeleno' plum treated with BC and NC were not significantly different as that of untreated plum after 35 d cold storage (Table 8.13). Whilst the phenolic contents of 'Angeleno' plum treated with NC fumigations and NC spray solution, regardless of MAP, were significantly reduced as compared to control after 50 d cold storage. Ascorbic acid concentrations of 'Angeleno' plum treated with NC were significantly lower up to 1.3 fold as compared to control after 35 d cold storage (Table 8.13). Antioxidant capacities of the fruit fumigated with BC and NC were significantly lowered as compared to control and spray solution treatments after 35 d cold storage (Table 8.13). However, ascorbic acid and antioxidant capacity of 'Angeleno' plum were not significantly affected by the ethylene antagonists after 50 d cold storage. Total phenols, ascorbic acid and antioxidant capacity of 'Angeleno' plum stored with MAP were not significantly different as the fruit without MAP in both cold storage periods. There was no interaction effect of treatments and storage type on these parameters of 'Angeleno' plum in both cold storage periods (Table 8.13).

Total phenolic contents of 'Flavor Fall' pluot treated with ethylene antagonists BC, NC and 1-MCP were significantly lowered up to 1.2 fold after 40 d cold storage irrespective of the treatments (Table 8.14). Phenolic content of the fruit fumigated with BC and 1-MCP were significantly lowered as compared to control and other treatments after 60 d cold storage (Table 8.14). The amount of ascorbic acid in the fruit treated with ethylene antagonists (BC, NC and 1-MCP), except BC and NC spray solutions, were maintained significantly lower as compared to control after 40 d cold storage (Table 8.14). The amount of ascorbic acid in the fruit fumigated with NC and 1-MCP were maintained significantly lower (1.1 fold each) as compared to control and the other treatments after 60 d cold storage. Antioxidant capacity of 'Flavor Fall' pluot treated with ethylene antagonists were not significantly different as compared to control after 40 d and 60 d cold storages (Table 8.14). Total phenols, ascorbic acid and the antioxidant capacity of 'Flavor Fall' pluot stored with MAP were not significantly different from the fruit without MAP after 40 d and 60 d cold storages, except phenolic content of pluot stored with MAP for 60 d in cold storage which was significantly lower than that of the fruit without MAP (Table 8.14). There was no interaction effect

between treatments and storage type on total phenols, ascorbic acid and antioxidant capacity of the pluot stored for 40 d and 60 d at 1°C (Table 8.14).

Table 8. 13 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on total phenols, ascorbic acid and antioxidant capacity of 'Angeleno' plum stored at 1°C for 35 d and 50 d.

Treatments	Storage period (d)					
	35 d			50 d		
	MAP	No MAP	Mean(Tr.)	MAP	No MAP	Mean(Tr.)
	Total phenols (g GAE kg^{-1})					
Control	72.36±6.705	63.62±4.587	67.99	91.32±5.260c	90.19±6.339c	90.76C
BCS	56.89±2.314	56.26±3.574	56.57	95.43±7.083c	93.06±3.802c	94.25C
BCF	63.50±9.235	71.23±3.727	67.37	72.60±3.620b	86.58±5.772c	79.59B
NCS	62.50±3.976	66.99±0.769	64.75	68.99±2.434ab	67.37±3.267ab	68.18A
NCF	56.26±1.870	59.63±0.620	57.95	54.14±0.269a	63.87±2.127ab	59.01A
Mean(MAP)	62.30	63.55		76.5	80.21	
LSD (P≤ 0.05)	Tr=ns	st=ns	Tr.st=ns	Tr=9.83**	st=ns	Tr.st =ns
	Ascorbic acid (mg kg^{-1})					
Control	23.29±0.791	22.54±0.568	22.91C	22.54±0.660	22.45±0.033	22.49
BCS	22.35±0.333	22.07±0.536	22.21C	21.26±0.429	20.65±1.041	20.96
BCF	22.70±1.448	20.37±0.133	21.54BC	19.67±0.149	19.95±0.537	19.81
NCS	17.16±0.564	17.48±0.914	17.32A	23.26±1.569	20.09±0.399	21.68
NCF	20.30±1.106	20.02±0.636	20.16B	22.42±1.724	22.38±0.421	22.40
Mean(MAP)	21.16	20.50		21.83	21.10	
LSD (P≤ 0.05)	Tr=1.92**	st=ns	Tr.st=ns	Tr=ns	st=ns	Tr.st=ns
	Antioxidant capacity (mmol TEAC kg^{-1})					
Control	174.1±12.30	160.1±8.548	167.1B	171.2±5.062	176.0±1.483	173.6
BCS	175.1±7.709	167.1±13.09	171.1B	164.7±15.01	196.8±8.411	180.8
BCF	141.1±4.879	151.7±8.028	146.4A	152.3±13.00	163.9±4.891	158.1
NCS	173.5±6.176	159.6±11.07	166.6B	164.7±3.085	165.3±2.171	165.0
NCF	138.1±1.389	153.2±11.11	145.6A	163.1±3.511	164.4±4.149	163.8
Mean(MAP)	160.39	158.33		163.23	173.26	
LSD (P≤ 0.05)	Tr=19.42*	st=ns	Tr.st=ns	Tr=ns	st=ns	Tr.st=ns

n = 3 replicates (12 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP), ns=non-significance. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 35 d and 50 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

Table 8. 14 Effect of ethylene antagonists applied as spray solutions (BCS and NCS) and fumigations (BCF and NCF) with or without MAP on total phenols, ascorbic acid and antioxidant capacity of 'Flavor Fall' pluot stored at 1°C for 40 d and 60 d.

Treatments	Storage period (d)					
	40 d			60 d		
	MAP	No MAP	Mean (Tr.)	MAP	No MAP	Mean (tr.)
	Total phenols (g GAE kg^{-1})					
Control	60.6±5.1	67.1±5.6	63.9B	82.5±3.9ab	95.7±8.3b	89.1B
BCS	52.5±2.2	54.9±3.7	53.7A	89.7±3.4ab	89.5±4.4ab	89.6B
BCF	52.3±2.3	57.8±4.3	55.0A	76.2±1.6a	78.3±3.1a	77.3A
NCS	53.0±3.0	55.9±0.7	54.5A	79.6±3.2a	89.2±6.5ab	84.4AB
NCF	52.8±3.1	60.5±3.6	56.6A	86.3±5.3ab	74.1±5.7a	80.2AB
1-MCP	56.5±1.1	54.0±2.3	55.4A	75.4±6.5a	74.2±2.4a	74.8A
Mean (st)	54.6A	58.4B		81.6	83.5	
LSD (P≤ 0.05)	st=3.6*	Tr=6.2*	Tr.st=ns	st=ns	Tr=9.9*	Tr.st=ns
	Ascorbic acid (mg kg^{-1})					
Control	15.4±0.5	16.7±0.9	16.1B	19.4±0.3	19.3±0.1	19.4C
BCS	14.8±0.3	15.1±0.2	14.9A	18.0±0.5	20.2±0.8	19.1BC
BCF	14.6±0.3	14.8±0.5	14.7A	19.0±0.5	18.7±0.5	18.9BC
NCS	16.4±0.2	16.0±0.3	16.2B	19.0±0.1	18.3±0.1	18.7ABC
NCF	14.8±0.1	16.3±0.2	15.5AB	17.9±0.1	18.1±0.7	18.0AB
1-MCP	16.3±0.6	16.8±0.1	16.5B	17.7±0.1	17.4±0.3	17.6A
Mean (st)	15.4	15.9		18.52	18.67	
LSD (P≤ 0.05)	Tr=0.98*	st=ns	Tr.st=ns	Tr=1.1*	st=ns	Tr.st=ns
	Antioxidant capacity (mmol TEAC kg^{-1})					
Control	126.9±1.1	133.5±4.1	130.2	170.2±2.7cd	155.6±8.3abc	162.9
BCS	138.2±2.6	149.4±4.5	143.8	162.7±7.0bc	155.8±6.8abc	162.9
BCF	138.5±7.8	134.8±6.5	136.6	159.3±11.3abc	142.4±1.5abc	150.8
NCS	136.8±2.4	138.6±2.6	137.7	154.9±10.3abc	157.6±6.8abc	156.3
NCF	134.3±4.3	146.4±8.6	140.4	143.8±13.1abc	137.5±3.3ab	140.6
1-MCP	134.0±2.4	145.5±1.2	139.8	130.4±4.4a	193.0±7d	161.7
Mean (st)	134.8	141.4		153.5	157.0	
LSD (P≤ 0.05)	Tr=ns	st=ns	Tr.st=ns	Tr=ns	st=ns	Tr.st=27*

n = 3 replicates (12 fruit per replication), Data are means ± SE. Tr=treatments, st=storage type (MAP or No MAP), ns=non-significance. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). The data for 40 d and 60 d cold storage were analysed separately. Mean values followed by a similar letter are not significantly different within the columns. **, * = significant at 1% and 5% level of LSD, ns=non-significance.

8.4 Discussion

8.4.1 Ethylene production

The ethylene antagonists BC, NC and 1-MCP were effective in delaying and suppressing the ethylene production of 'Angeleno' plum and 'Flavor Fall' pluot under cold storage up to 50 d and 60 d when they were applied as fumigants. The reduction in ethylene production of the fruit treated with BC, NC and 1-MCP is consistent with the irreversible binding of ethylene antagonists to the ethylene receptors as proposed by Sisler and Serek (1997) and Pirrung et al., (2008). Similarly, ethylene production was suppressed by the fumigation of 1-MCP (1 µL L^{-1}), when it was applied as pre-storage treatment to 'Tegan Blue' plum stored at 0±1°C up to 6 weeks (Khan and Singh, 2008) and applied as post-storage treatment to 'Shushanggan' apricot stored at -1.9±0.2°C for 30 d (Fan et al., 2018). In both 'Angeleno' plum and 'Flavor Fall' pluot, the spray solutions of BC and NC were less effective in comparison to the fumigation treatments, although they both tendentially showed better outcomes than the control. This was likely due to incomplete diffusion of BC and NC to the ethylene receptors throughout the fruit when they were applied as spray solutions. This assumption is supported by Sisler et al. (2009) who required larger amounts of cyclopropene salts solution and the longer exposure time to prevent the ethylene action in banana ripening. The limited ability of these cyclopropene salts solution to diffuse through the fruit compared to the gaseous cyclopropene was thought to be the cause of that observation. In the case of the plum and pluot stored with MAP, the reduction in ethylene peak concentration and the delayed peak onset is attributed to the increased carbon dioxide concentration inside the MAP bags (Brandenburg and Zagory, 2009; Valero and Serrano, 2010b). Carbon dioxide is a natural inhibitor of the responses to ethylene by suppressing the ethylene biosynthesis (Sisler and Wood, 1988; Sisler et al., 2006). In 'Flavor Fall' pluot, 1-MCP and BC performed better in delaying the ethylene peak onset with the presence of MAP in both cold storage periods. The synergistic effect of ethylene antagonist 1-MCP (1000 nL L^{-1}) and MAP on extending the storage life of 'Gros Michel' banana under cold storage (14°C) has been reported by Ketsa et al. (2013), concluding that the combined treatment of 1-MCP and MAP was more effective than the untreated or treated with one of them alone. A similar finding was recorded by Erkan and Eski (2012) in 'Autumn Giant' and 'Black Beauty' plums treated with 1-MCP (500 ppb) and MAP stored at 0°C for 60 d.

8.4.2 Respiration rate

The response of respiration rate to the ethylene antagonist in plums is dependent on the ripening behaviour of individual cultivars. Minas et al. (2015) revealed that the respiration rates of suppressed-climacteric 'Angeleno' and non-climacteric 'Sweet Miriam' plums were not influenced by 1-MCP (0.5 µL L^{-1}) whilst the respiration rate of climacteric 'Joanna Red' plum was suppressed by 1-MCP under similar storage conditions. Consistent to these reports, the respiration rate of 'Angeleno' plum and 'Flavor Fall' pluot in the present study did not respond to all the ethylene antagonists tested (BC, NC and 1-MCP) irrespective of the cold storage periods. Similarly, the respiration rate of the 'Red Rosa' plum treated with 0.1 ppm 1-MCP for 20 h and stored at 0°C for 5 weeks was not different from that of control (Dong et al., 2001). The 'Blackamber' plum treated with different concentrations of 1-MCP and stored at 0°C for up to 45 d did not show any significant difference in respiration rate from the control fruit (Ozkaya et al, 2010). Regardless of the cold storage periods, the MAP neither delayed nor reduced the respiration peak onsets and peak rates of 'Angeleno' plum and 'Flavor Fall' pluot. The MAP generates the atmospheric condition of increased carbon dioxide and decreased oxygen concentrations inside the package caused by the respiration of the produce. These modified atmosphere conditions reduce the respiration rate (Valero and Serrano, 2010b). In the present study, the negligible effect of MAP on the respiration rate, which was determined as carbon dioxide production, of 'Angeleno' plum and 'Flavor Fall' pluot may presumably be due to their suppressed-climacteric nature. The suppressed-climacteric cultivars produce a relatively low amount of CO_2 as compared to the climacteric plums (Abdi et al, 1997).

8.4.3 Weight loss and firmness

The BC and NC formulations reduced the weight loss of 'Angeleno' plum stored for 50 d at 1°C which is consistent to the previous findings in climacteric plum 'Santa Rosa' and non-climacteric plum 'Golden Japan' (Martinez-Romero et al., 2003). The weight loss of 'Angeleno' plum stored for 35 d and 'Flavor Fall' pluot stored for 40 d and 60 d did not respond to the ethylene antagonists tested (BC, NC and 1-MCP). Similarly, the 'Blackamber' plum treated with different concentrations of 1-MCP maintained the same weight loss percentage as control stored at 0°C for 15, 30 and 45 d (Ozkaya et al, 2010). The higher fruit weight retention up to six-fold for 'Angeleno'

plum and ‘Flavor Fall’ pluot stored with MAP was the result of increased water vapour pressure inside the MAP bags. The MAP films restrict the water vapour diffusion across the bags and maintain the saturated vapour pressure to slow down the transpiration rate of the fruit resulting in weight loss reduction (Valero and Serrano, 2010b). Similar reduction in weight loss has been reported in ‘Tegan Blue’ plum stored with LifeSpan MAP bags (Khan and Singh, 2008), in ‘Friar’ plum stored with LifeSpan L316 box liner films (Cantin et al., 2008), in ‘Sanacore’ and ‘Ariddo di Core’ plums stored with different types of MAP films (Sottile et al., 2013) and in ‘Angeleno’ plum stored under active MAP condition (Peano et al., 2017).

All the ethylene antagonists tested in either spray solutions or fumigation treatments, maintained significantly higher firmness in ‘Angeleno’ plum up to 35 d and in ‘Flavor Fall’ pluot up to 40 d at 1°C. However, only fumigation treatments, regardless of MAP, were effective in maintaining the firmness of ‘Angeleno’ plum after 50 d cold storage and ‘Flavor Fall’ pluot after 60 d cold storage. The ethylene antagonist 1-MCP was also found to retain the fruit firmness of climacteric plum ‘Santa Rosa’ and non-climacteric plum ‘Golden Japan’ stored at 1°C (Martinez-Romero et al., 2003). The firmness retention is attributed to the suppression of the activities of fruit softening enzymes, polygalacturonases (PGs), by reducing ethylene production. Ethylene up regulates the activities of PGs and this has been demonstrated in ethylene treated ‘Akatsuki’ peach (Hayama et al., 2006b). The higher fruit firmness retention by ethylene suppression was reported in 1-MCP treated ‘Tegan Blue’ plum (Khan and Singh, 2007a) and ‘Maria Aurelia’ nectarine (Ozkaya et al., 2016). The firmness of ‘Angeleno’ plum and ‘Flavor Fall’ pluot stored with MAP were higher as compared to the fruit without MAP due to the carbon dioxide rich atmosphere inside the bags which leads to slow down the activity of PGs enzymes which are responsible for fruit softening (Ozkaya et al. 2016). The BC and NC spray solutions performed better in combination with MAP than without MAP in maintaining fruit firmness. Similarly, the combined treatment of 1-MCP and MAP maintained higher firmness in “Gros Michel’ banana as compare to the fruit treated with either of them alone (Ketsa et al., 2013).

8.4.4 SSC, TA and SSC:TA

In both cultivars tested, the effectiveness of ethylene antagonists in maintaining SSC, TA and SSC:TA is highly dependent on MAP, especially after a long cold storage period. The ethylene antagonists performed better in the presence of MAP. The lower SSC percent and SSC:TA, and higher TA percent in the fruit treated with ethylene antagonists is ascribed to the reduction in ethylene production in these fruit slowing down the ripening related processes. The similar results were reported in 'Songold' plum treated with 1-MCP (0.6 µL L^{-1}) before cold storage (Velardo-Micharet et al., 2017) and in 'Shushangan' apricot treated with 1-MCP after cold storage at -1.9±0.2°C for 30 d (Fan et al., 2018). In both 'Angeleno' plum and 'Flavor Fall' pluot, MAP showed significant effect in maintaining lower SSC percent and SSC:TA ratio, higher TA percent, except SSC:TA ratio of 'Angeleno' plum after 50 d cold storage and SSC:TA ratio of 'Flavor Fall' pluot after 40 d cold storage. This might be the consequential effect of reduction in ethylene production of MAP-stored fruit with the delayed ripening processes. A similar reduction in SSC content by MAP was reported in 'Maria Aurelia' nectarine (Ozkaya et al., 2016), in 'Tegan Blue' plum (Khan and Singh, 2008).

8.4.5 Individual sugars and organic acids

During fruit growth and ripening of Rosaceae family members, sugar accumulation is mainly in the form of sucrose and sorbitol (Valero and Serrano, 2010c). The breakdown of sucrose to glucose and fructose is intensified while the accumulation of organic acids is declined during fruit ripening of plum (Singh et al., 2009; Valero and Serrano, 2010c). The higher sucrose and sorbitol concentrations and lower glucose and fructose concentrations in 'Angeleno' plum and 'Flavor Fall' pluot fumigated with BC, NC and 1-MCP are ascribed to the delayed ripening process through suppression of ethylene production. Similarly, Fan et al. (2018) reported that the apricot treated with 1-MCP showed significantly higher sorbitol and comparatively higher sucrose and lower glucose and fructose concentrations as compared to control and the fruit treated with ethylene. The influence of ethylene on sugar accumulation during fruit ripening was proposed by Defilippi et al. (2004) in apple and by Chervin et al. (2006) in grape berries using the ethylene antagonist 1-MCP. Similarly, Kim et al. (2015) also observed the interaction between the ethylene and sugar accumulation during fruit

ripening through regulation of sorbitol synthesis by 1-MCP in ‘Santa Rosa’ and ‘Sweet Miriam’ plums. In the present study, malic acid was found to be the predominant organic acid in both ‘Angeleno’ plum and ‘Flavor Fall’ pluot, as previously reported in ‘Black Amber’, ‘Amber Jewel’ and ‘Angeleno’ plums by Singh and Singh (2008) and Singh et al. (2009). Fumigation with ethylene antagonists BC, NC and 1-MCP significantly maintained higher malic acid contents in ‘Angeleno’ plum after 35 d and 50 d cold storage and in ‘Flavor Fall’ pluot after 40 d cold storage as in agreement with the previous report of Fan et al. (2018) in ‘Shushanggan’ apricot. The MAP maintained higher sucrose and malic acid levels in ‘Angeleno’ plum and ‘Flavor Fall’ pluot indicating the delayed fruit ripening process (Valero and Serrano, 2010b).

8.4.6 Total phenols, ascorbic acid and antioxidant activity

The response of total phenols, ascorbic acid and antioxidant activity to the ethylene antagonists were varied depending on the cultivars and cold storage periods. The total phenol contents and antioxidant activity in ‘Angeleno’ plum and ‘Flavor Fall’ pluot were observed to increase with the long cold storage period as previously reported in different cultivars of plums by Daiz-Mula et al. (2009 and 2011b). The reduction in phenolic contents, ascorbic acid and antioxidant activity in the fruit fumigated with ethylene antagonists is likely due to the lower accumulation of sugars, especially glucose and fructose. Pirie and Mullins (1977) reported that sugars have a regulatory role in the biosynthesis of anthocyanins and polyphenols in grape berries. The significant correlation between total sugar accumulation and the content of total phenols, ascorbic acid and antioxidant activity was also reported in nectarines (Abdi et al., 2011). ‘Angeleno’ plum and ‘Flavor Fall’ pluot stored with MAP retained the same concentrations of total phenols, ascorbic acid and antioxidant activity as of the fruit without MAP irrespective of cold storage period. The relatively lower phenolic contents in the fruit stored with MAP is thought to be due to the delayed ripening processes induced by MA condition as reported in different yellow and purple plum cultivars (Daiz-mula et al., 2011b).

8.5 Conclusion

The fumigation with BC, NC and 1-MCP suppressed ethylene production and preserved the postharvest fruit quality of cold-stored ‘Angeleno’ plum and ‘Flavor Fall’ pluot fruit up to 50 and 60 d respectively, regardless of MAP. However, the effectiveness of BC and NC spray solutions on ethylene production and other fruit quality parameters was more pronounced when the fruit were stored with MAP. The MAP substantially reduced ethylene production, physiological weight loss and maintained the other fruit quality attributes of ‘Angeleno’ plum and ‘Flavor Fall’ pluot during the respective cold storage periods. The levels of total phenols, ascorbic acids and the total antioxidant activities in the plum and pluot fruits treated with ethylene antagonists were retained similar to those of the untreated plums, irrespective of MAP. The interaction effect between the ethylene antagonists and the MAP on ethylene production and the quality parameters were different depending on the fruit type and cold storage periods. In conclusion, the storage life of ‘Angeleno’ plum and ‘Flavor Fall’ pluot can be prolonged up to 50 and 60 d, respectively by the applications of ethylene antagonists as fumigants alone or in combination with MAP.

CHAPTER 9

General discussion, conclusion and future prospects

9.1 General discussion

In Australia, almost eighty percent of the total production of peach, nectarine and plum are supplied as fresh fruit to both domestic and export markets (Horticulture Innovation Australia Limited, 2017). The consumption of fresh stone fruit is increasing due to their attractive appearance, sweet taste and flavour, and the abundance in bioactive compounds with health benefit properties (Crisosto et al., 2006; Vinholes et al., 2016). Therefore, it is important to maintain the eating, aesthetic and nutritional quality of the fruit throughout the food supply chain (Kader, 2008). Unfortunately, fresh peach, nectarine and plum fruits cannot be stored for long periods without affecting the overall fruit quality. They are highly perishable and overripen within a week after harvest under ambient conditions which limits their potential marketable life and increases postharvest losses (Crisosto and Day, 2012; Khan et al., 2018).

Ethylene is one of the key factors responsible for the spoilage of fruits and vegetables along the supply chain (Blanke, 2014). It triggers fruit ripening and then accelerates the senescence processes leading to the deterioration of fruit quality and reduction in postharvest storage life (Burg and Burg, 1965; Kader, 1985). Stone fruit are climacteric fruit, however, some plum cultivars display suppressed-climacteric features, and produce a relatively large amount of ethylene at the initial stage of fruit ripening (Tonutti et al., 1991; Abdi et al., 1997). This ethylene is responsible for the irreversible changes in postharvest quality attributes such as fruit softening associated with stone fruit ripening (Bapat et al., 2010). Appropriate postharvest management approaches, alongside the technologies to inhibit ethylene production, is the key to postpone fruit ripening and to preserve overall fruit quality (Zhang et al., 2017; Khan et al., 2018).

Cold storage has been a fundamental practice to extend postharvest life and to prevent quality deterioration of stone fruit (Crisosto et al., 1995). Lowering storage temperature can effectively slow down the metabolic processes of the stored fruit resulting in delayed fruit ripening (Valero and Serrano, 2010c). However, the stone fruit stored for long periods at low temperature are vulnerable to chilling injury

(Crisosto et al., 1995). Inhibiting ethylene production (Candan et al., 2008) and precise management of Low-temperature storage (0-2 °C) can reduce the incidence of chilling-induced physiological disorder during postharvest storage of stone fruit, e.g. in 'Larry Ann' and 'Friar' plum (Wang et al., 2016).

Modified atmosphere packaging (MAP) is another postharvest management practice to extend storage life and to assure quality maintenance of the fresh fruits (Valero and Serrano, 2010b). The lower oxygen and higher carbon dioxide atmosphere inside the MAP can slow down the respiration rate of fruit and thus the subsequent metabolic processes (Brandenburg and Zagory, 2009). The MAP technology has been reported as a promising tool in extending the storage life and quality maintenance of stone fruit during postharvest handling, e.g. in peach, nectarine (Akbudak and Eris, 2004) and plum (Peano et al., 2017). Despite the effectiveness in hindering the metabolic process of harvested fruit, managing the storage temperature and atmosphere alone are less likely to control the production and effects of ethylene during storage.

A several number of technologies have been developed to prevent the detrimental effect of ethylene during postharvest handling of fresh fruit. These technologies include inhibiting the biosynthesis, removing the ethylene present in the storage atmosphere and blocking the reception of ethylene at the receptor level (Zhang et al., 2017). Inhibiting the action of ethylene by blocking the receptors with the gaseous ethylene antagonist, 1-methylcyclopropene (1-MCP) has been recommended as the most effective way to prevent ripening and senescence of horticultural crops during storage (Watkins, 2006; Valero et al., 2016). The aqueous application of 1-MCP has also been reported as an alternative to fumigation treatment in conditions where the proper facilities are not available or in packing-line treatments as dip or spray (Watkins, 2015). However, the required amount of 1-MCP for an aqueous solution is 700-fold higher than the gaseous 1-MCP (Argenta et al., 2007). In addition, the efficacy of aqueous 1-MCP is lost when the application is followed by another technology, such as the application of sodium hypochlorite solutions (Choi et al., 2009). The recently developed ethylene antagonists, 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) are proposed to be an alternative to 1-MCP with the potential properties for preparation of aqueous solutions (Singh et al., 2018). However, no research work has been reported on the aqueous formulations of BC and NC to date.

The use of adjuvants, any substances which can promote the performance of the active ingredient or increase the contact area of a solution and target plant parts (Somervaille et al., 2012), has been reported as a prerequisite practice in preparation of aqueous solutions. The application of non-ionic surfactant, Tween® 20, in preparation of calcium chloride dip treatment has improved calcium uptake in mango fruit (Singh et al., 2000). The co-solvent such as ethanol has been reported not only as a solubility promoter for partially water soluble compounds (Grichko, 2006) but as a penetration enhancer through the fruit cuticles of cranberry for the field application of ethephon solution (Farag et al., 1992). The cyclodextrins, α and β cyclodextrins are complexation compounds used in the commercial products of 1-MCP such as Ethylblock®, SmartFresh™ and SmartTabs™ to preserve the release of 1-MCP for longer time (Sisler et al., 2006; Valero et al., 2016).

The present research was designed to study the influence of different aqueous formulations of BC and NC on fruit ripening and postharvest fruit quality during storage conditions which are commonly practised by the stone fruit industry. Tween® 20, ethanol and β-cyclodextrin were used as adjuvants for the preparation of the tested aqueous solutions. It was hypothesised that the different formulations of 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) would suppress ethylene production of peach, nectarine and plum fruit. The ripening of these treated fruits would be postponed and the overall fruit quality would be maintained during postharvest storage. In addition, the best adjuvant for the preparation of aqueous solutions of BC and NC would be uncovered. Ethylene production, respiration rate, weight loss, fruit firmness, soluble solids content (SSC), titratable acidity (TA), SSC:TA, individual sugars (glucose, sucrose, fructose and sorbitol), individual organic acids (malic, citric, succinic and fumaric), ascorbic acid content, total phenolic content and total antioxidant capacity were evaluated in all the conducted experiments. The five research experiments: (1) screening of BC formulations, (2) screening of NC formulations, (3) optimizing the formulations of BC and NC, (4) standardizing the concentration of the best adjuvant, ethanol and (5) examining the synergistic effect of BC and NC with MAP, the most commonly used complementary storage method in Australia stone fruit industry, were conducted to fulfil the major aim of this study. The outcomes of each experiment are briefly discussed in the following sections. However, it is important to note that postharvest fruit quality and behaviour are largely

influenced by pre-harvest factors (Manganaris et al., 2008). The fruit size, colour, sugar and acid accumulation and compositions of bioactive compounds during fruit development and maturation are varied depending on temperature, light intensity, carbon availability and water availability during the pre-harvest conditions (Lechaudel and Joas, 2007; Arah et al., 2015). The response of stone fruit to BC and NC treatments is likely to be different depending on their growing environment.

9.1.1 Effects of five different formulations of 1*H*-cyclopropabenzene (BC) on plums

This experiment was conducted to screen the most potential formulations of BC. The five different formulations of BC: aqueous solution of BC prepared with only distilled water, aqueous solution of BC prepared with 0.02 % Tween® 20, aqueous solution of BC prepared with 5 % β-cyclodextrin, aqueous solution of BC prepared with 5 % ethanol and fumigation of BC were tested on 'Fortune', 'Angeleno' and 'Tegan Blue' plums stored for 25 and 40 days at 0±1°C (90±5% RH). The results indicated that ethylene production of 'Fortune', 'Angeleno' and 'Tegan Blue' plums were suppressed with the application of different formulations of BC, regardless of the adjuvants and cold storage period. BC might have blocked the action of ethylene by interacting with the copper cofactor of the ethylene receptor as previously reported in 1-MCP (Sisler, 2006, Pirrung et al., 2008). The proposed mechanism of ethylene receptor binding reaction for BC is described in section 2.2.5.2 (Fig. 2.8). The extent of ethylene suppression was greater with the BC fumigation treatment followed by the aqueous solution of BC prepared with ethanol and the aqueous solution of BC prepared with Tween® 20. Fruit firmness was higher and physiological weight loss was lower in these treatments, as well. In agreement with our results, Sisler et al., (2009) also reported that fumigation of cyclopropenes was more effective as compared to the aqueous salt solutions of cyclopropenes in delaying fruit ripening of banana. The respiration rate of all the cultivars did not respond to BC regardless of formulations and storage period. Anthocyanin accumulation was reduced in the BC-treated fruits. In general, the fruit quality attributes were not significantly affected by the BC formulations, although the responses were varied depending on the cultivar. The adjuvants ethanol and Tween® 20 improved the performance of aqueous solutions of BC. This might have attributed to the amphiphilic molecular structure of adjuvants, ethanol and Tween® 20, which have the ability to increase the solubility of poorly

water soluble compounds and the permeability of cuticle (Farag et al., 1992; Somervaille et al., 2012). Overall, BC can be applied, either as fumigation or as spray solutions in the presence of ethanol or Tween® 20, as a postharvest treatment to delay fruit ripening and the related quality changes of 'Fortune', 'Angeleno' and 'Tegan Blue' plums.

9.1.2 Effects of five different formulations of 1*H*-cyclopropa[*b*]naphthalene (NC) on plums

This experiment was conducted to screen the most potent formulations of NC. The five different formulations of NC were aqueous solution prepared with only distilled water, an aqueous solution prepared with 0.02 % Tween® 20, an aqueous solution prepared with 5 % β-cyclodextrin, an aqueous solution prepared with 5 % ethanol and fumigation. They were tested on 'Angeleno' and 'Tegan Blue' plums stored for 25 and 40 days at 0±1°C (90±5% RH). The NC formulations, irrespective of adjuvants, significantly suppressed ethylene production of 'Angeleno' and 'Tegan Blue' plums in both storage periods. Reduction in physiological weight loss, softening, soluble solids content along with higher accumulations of TA and organic acid contents were observed in the plums treated with NC. The antagonistic property of NC in binding the ethylene receptor, as depicted in section 2.2.5.2, (Fig. 2.9), might have suppressed production of ethylene and delayed the subsequent ripening processes (Sisler, 2006; Watkins, 2015). However, the degree of significance in the effectiveness of NC on these parameters were varied depending on the formulations and the cultivars. The NC fumigation and NC aqueous solutions with ethanol or Tween® 20 outperformed the other formulations. The better performance of NC fumigation treatment may be due to the easy diffusion of gaseous NC through the stomata of fruit surface coupled with the longer exposure time (Sisler et al., 2009). The presence of adjuvants, ethanol or Tween® 20 might have enhanced the solubility (Grichko, 2006) and infiltration of NC through the fruit cuticle (Otero-Diaz et al., 2017) leading to the greater performance of these solutions compared to aqueous solutions without adjuvants as described in section 5.4.1 (Fig. 5.3). All the NC formulations maintained the levels of total phenols, ascorbic acid, total anthocyanin and the total antioxidant capacity in treated 'Angeleno' and 'Tegan Blue' plums similar to that of the untreated plums. These results revealed that NC can be applied as a fumigant or as a spray solution to

manipulate fruit ripening and to preserve the overall postharvest fruit quality of ‘Angeleno’ and ‘Tegan Blue’ plums up to 40 d under cold storage. The presence of ethanol or Tween® 20 in NC aqueous solutions was found to enhance the performance of NC aqueous solutions.

9.1.3 Effects of different formulations of BC and NC on peach, nectarine and plum

The six most effective formulations of BC and NC: fumigations of BC and NC, aqueous solutions of BC and NC prepared with 5% ethanol, aqueous solutions of BC and NC prepared with 0.02% Tween® 20 based on the previous results, were evaluated to optimize the formulations of BC and NC. The aqueous solutions prepared only with either ethanol or Tween® 20 or ethanol plus Tween® 20, and without any treatment were examined as controls. All the treatments were tested on cold-stored ‘Princess Time’ peach, ‘Diamond Bright’ nectarine and ‘Tegan Blue’ plum to address the third objective of this study. The fumigations with BC and NC substantially reduced ethylene production and the weight loss, while maintaining higher fruit firmness of the tested peach, nectarine and plum under ambient (20±1 °C and 85±5 %RH) or cold storage (1 °C and 90±5 % RH). It can be assumed that gaseous BC and NC could easily diffuse to the target cell where the ethylene receptors are located through the stomata in the fruit surface as postulated in Fig. 6.9 (A) in section 6.4. The comparative longer exposure time in fumigation treatments (18 h) than the aqueous treatments (≈ 5-10 min) might also be the reason of the better performance of BC and NC fumigation (Sisler et al., 2009). For aqueous solutions, variation in the effectiveness of BC and NC on ethylene production was observed depending on the species, the storage condition and the adjuvants (Watkins, 2006 and 2015). In ‘Princess Time’ peach, the NC aqueous solution prepared with ethanol maintained significantly lower ethylene production as compared to control and the ethanol only aqueous solution after 25 d cold storage (Fig. 6.1 A and B). In ‘Diamond Bright’ nectarine, all the aqueous solutions irrespective of the presence of adjuvants and the ethylene antagonists exhibited the significantly lower ethylene production after 25 d cold storage (Fig. 6.2 A and B). In ‘Tegan Blue’ plum, the aqueous solutions of BC and NC, regardless of the adjuvants applied, significantly suppressed ethylene production compared to the controls under ambient condition (Fig. 6.3 A and B). The proposed mechanism of

aqueous solutions of BC and NC with adjuvants were described in Fig. 6.9 (C), section 6.4. Similarly, the response of quality attributes to BC and NC were observed to be different depending on the formulations and fruit species. These variations in the effectiveness of the aqueous solutions of BC and NC might be due to differences in morphological structures of fruit surface in peach, nectarine and plum. Fruit surface, being the direct contact medium to spray solutions, presumably determines the infiltration rate. The epicuticular composition of fruit are varied depending on the species (Koch and Ensikat, 2008) and the surface cuticles influence the postharvest quality and storage life of fruits (Martin and Rose, 2014; Lara et al., 2014). In addition, the different functional properties of adjuvants, ethanol and Tween® 20 (Farag et al., 1992; Somervaille et al., 2012) might also be ascribed to the variation in efficacy of BC and NC by affecting the uptake of BC and NC aqueous solutions by the tested fruits.

9.1.4 Effects of different concentrations of ethanol on the performance of aqueous solutions of BC and NC

Ethanol was consistently the most effective adjuvant in enhancing the performance of aqueous solutions of BC and NC in the previous experiments, so the ethanol adjuvant was explored in more detail. Farag, et al., (1992) reported that ethanol enhanced the uptake of ethephon spray solution in cranberry fruit by increasing the cuticle permeability. The effect of different concentrations of ethanol (2.5, 5.0, 10 %) on aqueous solutions of BC and NC, along with the fumigation of BC and NC, were examined using ‘Ruby Diamond’ nectarine and ‘Tegan Blue’ plum. All BC and NC formulations suppressed ethylene production by blocking the ethylene receptor, thereby impeding the subsequent actions of ethylene as postulated by Sisler (2006) and Pirrung et al, (2008). The addition of ethanol at the concentration of 2.5 and 5.0 % was observed to suppress ethylene production of ‘Ruby Diamond’ nectarine and ‘Tegan Blue’ plum, and the reduction of ethylene was significant only in ‘Tegan Blue’ plum (Fig. 7.1 and 7.2). The BC and NC aqueous solutions with 10 % ethanol exhibited higher ethylene production than control nectarine and plum fruit under ambient condition (Fig. 7.1 A and 7.2 A). This observation might have attributed to the higher concentration of ethanol which dissolves cuticle disrupting the barrier properties of fruit surface cuticle and causing more stress to the plum and nectarine fruit (Farag et al., 1992, Curry, 2008 and Lara et al., 2014). Fumigations with BC and NC suppressed

ethylene production of ‘Tegan Blue’ plum in both ambient and cold storage conditions, while the significant suppression in ethylene production of ‘Ruby Diamond’ nectarine was observed only under ambient condition (Table 7.1 and 7.2). The response of the fruit quality attributes to the BC and NC were varied depending on the concentration of ethanol and the fruit species as in agreement with the report of Watkins (2006). Overall, the aqueous solutions of BC and NC prepared with 5% ethanol not only suppressed ethylene production but also maintained the postharvest fruit quality of ‘Ruby Diamond’ nectarine and ‘Tegan Blue’ plum.

9.1.5 Synergistic effect of ethylene antagonists and modified atmosphere packaging (MAP) on plum and pluot

The effect of ethylene antagonists (BC, NC and 1-MCP) and the modified atmosphere packaging (MAP) to extend the cold storage life and to preserve fruit quality were investigated on ‘Angeleno’ plum and ‘Flavor Fall’ pluot. The ripening related fruit quality parameters were evaluated after the respective cold storage period, 35 d and 50 d for plum and 40 d and 60 d for pluot. The ethylene antagonists significantly lowered ethylene production of plum and pluot regardless of the cold storage period. The MAP also substantially reduced ethylene production of plum and pluot after the respective cold storage, except in ‘Angeleno’ plum stored for 50 d. The elevated carbon dioxide and decreased oxygen levels inside the MAP bags could slow down the metabolic process of fruit during storage (Valero and Serrano, 2010b). The effects of BC and NC spray solutions on ethylene production of plum and pluot were more prominent when in combination with MAP. The influence of ethylene antagonists on fruit firmness, weight loss, SSC, TA, sugars and organic acids were varied depending on the use of MAP, fruit type and the cold storage period. The fruit stored with MAP showed a substantial reduction in weight loss, regardless of fruit type and cold storage period (Watkins, 2006). The levels of total phenols, ascorbic acid and the total antioxidant capacity of plum and pluot treated with ethylene antagonists were maintained as in control fruit irrespective of MAP and cold storage. Overall, although the effectiveness of fumigation of ethylene antagonists was not dependent on MAP, the aqueous solutions of BC and NC performed better in combination with MAP in reducing ethylene production of plum and pluot. Similar synergistic effects of 1-MCP and MAP were reported in ‘Tegan Blue plum (Khan and Singh, 2008), in ‘Autumn Giant’ and ‘Black Beauty’ plums (Erkan and Eski, 2012) and in ‘Gros Michel’ banana (Ketsa et

al., 2013). With MAP, the aqueous solutions of BC and NC could preserve the fruit quality of ‘Angeleno’ plum and ‘Flavor Fall’ pluot up to 50 d and 60 d, respectively under cold storage.

9.2 Conclusion

- Fumigation with 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) was observed to be effective in reduction of ethylene production in all the tested stone fruits: ‘Princess Time’ peach, ‘Diamond Bright’ and ‘Ruby Diamond’ nectarines, ‘Fortune’, ‘Angeleno’ and ‘Tegan Blue’ plums, and ‘Flavor Fall’ pluot. The amount of ethylene reduced by the NC fumigation was comparatively higher as compared to that of BC fumigation in all the tested cultivars, indicating that NC is more promising in antagonising ethylene action. The degree of delaying the onset of climacteric ethylene peak was varied depending on the cultivars and the storage period.
- Aqueous solutions of BC and NC with the presence of ethanol have the highest potential to suppress ethylene production of plums and pluot. The concentration of ethanol can either be 2.5 % or 5.0 % in ‘Tegan Blue’ plum and ‘Ruby Diamond’ nectarine. The effectiveness of BC and NC on ethylene production of peach and nectarine were not consistent. The results indicated that the effectiveness of aqueous applications is somewhat dependent on the fruit surface morphology.
- The aqueous solutions of BC and NC with the presence of Tween® 20 or β-cyclodextrin exhibited variability in effectiveness to suppress ethylene production. Yet, Tween® 20 performed better in the plums and nectarines than in the peach.
- The ethylene antagonists did not show a significant effect on the respiration rate of all the tested cultivars. The effect of BC and NC, either fumigation or aqueous solutions, on the evaluated quality attributes in this study were observed to be varied in the cultivars and cold storage dependent manner. No adverse effect of BC and NC on the assessed fruit qualities was observed in all the tested cultivars.

- There was a positive synergistic effect between the ethylene antagonists (BC, NC and 1-MCP) and modified atmosphere packaging in suppressing ethylene production and quality maintenance of ‘Angeleno’ plum and ‘Flavor Fall’ pluot. With MAP, the ethylene antagonists can preserve the overall fruit quality of ‘Angeleno’ plum and ‘Flavor Fall’ pluot up to 50 d and 60 d respectively under cold storage, which is 15-20 days longer than the standard industrial storage practice.

9.3 Future prospects

- The effect of BC and NC on the activities of enzymes or the expression of genes which are responsible for ethylene production and fruit softening of stone fruit is required to fully understand the effect of BC and NC.
- Due to the variability of responses between species and cultivars observed in this study, the investigation on the effect of fumigation of BC and/or NC on other *Prunus* species such as cherry and apricot, as well as other horticultural crops, are warranted for the extensive postharvest applications of these ethylene antagonists.
- The field investigations on the aqueous solutions of BC or NC with different concentrations of ethanol could be useful for pre-harvest applications in plum production.
- The synergistic effect of BC and NC, and MAP is worthwhile to investigate on other *Prunus* species for storage life extension and quality preservation.

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Statement of contribution

To Whom It May Concern I, Poe Nandar Kyaw, 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. Mr Vijay Yadav Tokala has assisted me in conducting the experiments, the biochemical analysis and recording the data. I intend five papers to be published with the following titles:

1. Poe Nandar Kyaw, Zora Singh, Alan D. Payne and Vijay Yadav Tokala. Effect of different formulations of new ethylene antagonist BC on postharvest physiology and quality of plums cvs. 'Fortune, Tegan Blue and Angeleno' under cold storage.

2. Poe Nandar Kyaw, Zora Singh, Alan D. Payne and Vijay Yadav Tokala. Impact of postharvest spray and fumigation treatments of an anti-ethylene compound NC on physiology and quality parameters of plums cvs. 'Tegan Blue' and 'Angeleno'.

3. Poe Nandar Kyaw, Zora Singh, Alan D. Payne and Vijay Yadav Tokala. Effectiveness of ethylene antagonists BC and NC formulations with ethanol and Tween® 20 on production of ethylene and fruit quality parameters in peach, nectarine and plum.

4. Poe Nandar Kyaw, Zora Singh, Alan D. Payne and Vijay Yadav Tokala. Ethanol enhances the performance of ethylene antagonist solutions in retarding ethylene production and maintaining fruit quality of Japanese plums.

5. Poe Nandar Kyaw, Zora Singh, Alan D. Payne and Vijay Yadav Tokala. Extending the cold storage life of plum cv. 'Angeleno' and pluot cv. 'Flavor Fall' as affected by ethylene antagonists and modified atmospheric packaging.

Poe Nandar Kyaw

I, as a Co-Author, endorse that this level of contribution by the candidate indicated above is appropriate.

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