Department of Environment and Agriculture

Modulation of fruit ripening, storage time and quality of fruits with emulsion of Chitosan alone and loaded with Salicylic acid or Oxalic acid

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: ----------

Date: ----------

Dedication

To:

My father (Mohamed)

My mother (Zohra)

My uncle (Hamed)

My wife (Saada)

My sons and daughters

My brothers and sisters

For

"Their love, incredible patience and endless support during the entire period of my PhD study and throughout my life"

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Abstract

The use of chitosan, salicylic acid (SA) and oxalic acid (OA) alone as a coating has been reported to reduce postharvest losses and maintenance quality of different fruits. No research has however been reported on the effects of an emulsion of chitosan loaded with SA or OA on regulation of ethylene production, fruit softening, and quality parameters in climacteric and non-climacteric fruits. Therefore, investigations were carried out to determine if chitosan emulsion loaded with SA or OA is more effective than the application of chitosan, SA or OA individually in prolonging shef-life at ambient temperature and cold storage and maintaining quality of climacteric (nectarine and Japanese plum) and non-climacteric (sweet orange) fruits. The chitosan emulsion (1.5%) coating significantly ($P \leq 0.05$) suppressed the climacteric ethylene production, resulted higher level sucrose in ripe 'Honey Fire' nectarine fruit as compared to the control and all other treatments at ambient temperature. Highest level of firmness, soluble solids concentration (SSC), SSC: titratable acidity (TA) ratio, tartaric acid and vitamin C in ripe 'Honey Fire' nectarine fruit were recorded in the fruit coated with chitosan loaded with SA. In ripe 'Bright Pearl' nectarine fruit, the emulsion of chitosan treatment showed higher SSC:TA ratio, reduced loss of weight, higher level of fumaric acid, malic acid, succinic acid, tartaric acid and total organic acids, higher level of sucrose, fructose and total sugars as compared to the control and all other treatments. The fruit coated with chitosan emulsion loaded with SA exhibited suppressed ethylene production and highest firmness in 'Bright Pearl' nectarine fruit kept at ambient temperature.

Coating of chitosan loaded with SA was shown to be more effective in reducing ethylene production, and maintaining higher levels of fructose, malic acid and vitamin C in four-week cold stored 'Bright Pearl' nectarine fruit. The application of chitosan, SA or OA alone was more effective in maintaining various fruit quality parameters such as reducing loss of weight, firmness and disease incidence and increasing total organic acids, sugars and total antioxidants compared to the chitosan loaded with SA or OA . In conclusion, the 'Bright Pearl' nectarine fruit coated with chitosan emulsion, SA and OA alone were more effective in maintaining quality of four weeks cold stored fruit compared to chitosan emulsion loaded with SA or OA.

Chitosan emulsion coating suppressed ethylene production during ripening in both 'Tegan Blue' and 'Angelino' plums. In cultivar Tegan Blue the fruit coated with

chitosan emulsion loaded with SA exhibited lower weight loss and disease incidence, and higher levels of TA, total organic acids, total sugars, and vitamin C as compared to the uncoated fruit and coated with other coatings. ‘Angelino’ plum fruit coated with chitosan emulsion alone exhibited suppressed ethylene production, reduced loss of fruit firmness and disease incidence, and higher SSC:TA ratio, total organic acids, sugars and total antioxidants. Chitosan emulsion (1.5%) coating significantly suppressed mean ethylene production and reduced disease incidence compared to the control and other treatments in ‘Angelino’ plum fruit stored at cold condition. Chitosan emulsion (1.5%) loaded with SA suppressed mean ethylene production in ‘Tegan Blue’ plum fruit. Whilst, chitosan coating recorded higher level of TA, fructose, glucose, total sugars, level of citric acid, malic acid and total organic acids in ‘Angelino’ plum fruit. Higher level of firmness, sucrose and vitamin C and reduced weight loss and disease incidence compared to control and all other treatments in ‘Tegan Blue’ fruit were recorded with the combined treatment of chitosan and SA. There was a no specific trend in various fruit quality parameters in response to chitosan emulsion, SA and OA alone or chitosan emulsion loaded with SA or OA in sweet orange cv. Midknight Valencia. In general, chitosan, SA and OA alone was more effective in suppressing respiration rate and maintaining fruit firmness, SSC:TA ratio, Vitamin C, total antioxidants and reducing disease incidence as compared to the chitosan loaded with SA or OA. The proposed hypothesis that chitosan loaded with SA or OA will be more effective in maintaining fruit quality compared to the application of chitosan, SA or OA alone was proven in cv. Honey Fire nectarine fruit at ambient temperature and cv. Tegan Blue plum fruit kept at both ambient temberature and cold condition. Howevre, the proposed hypothesis was refuted in cv. Bright Pearl nectarine fruit and cv. Angelino plum fruit at both ambient temperature and cold storage and cv. ‘Midknight Valancia’ sweet orange fruit at cold condition.

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List of symbols and abbreviations

×	Multiply / interaction between
>	Greater than
≤	Less than or equal to
±	Plus minus
/	Divide
=	Equal
´	Minutes
°	Degree
°C	Degree celcious
%	Per cent
β	Beta
P	Picomole (s)
μ g	Microgram(s)
μ L	Microliter (s)
μ mol	Micromole(s)
1-MCP	1-Methylcyclopropene
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	1-aminocyclopropane-1-carboxylic acid oxidase
ACS	1-aminocyclopropane-1-carboxylic acid synthase
ADC	Arginine decarboxylase
a.i.	Active ingredient
ANOVA	Analysis of variance
AOA	Aminooxyacetic acid

AOC	Allene oxidase cyclise
AOS	Allen oxide synthase
APX	Ascorbate peroxidase
AVG	Aminoethoxyvinylglycine
Ca	Calcium
$CaCl_2$	Calcium chloride
C_2H_4	Ethylene
CI	Chilling injury
cm	Centimetre(s)
Co	Company
CO_2	Carbon dioxide / respiration
$CoSO_4$	Cobalt sulphate
conc.	Concentration
$CoCl_2$	Cobalt chloride
CPPU	N-(2-chloro-4-pyridyl)-N’-phenylurea
cv	Cultivar
d	Day(s)
dH_2O	Disttle water
DAFWA	Department of Agriculture and Food, Western Australia
DAS	Day after Spray
DAH	Day after harvest
DAT	Day after treatment
DPH	Day prior harvest
DPPH	2, 2-diphenyl-1-picryl-hydrazyl
DW	Dry powder
E	East

EGase	*Endo*-1,4- β-D-glucanase
Endo-PG	*Endo*-polygalacturonic acid
et al	At alia
Exo-PG	*Exo*-polygalacturonic acid
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation Statistics
$FeSO_4$	Ferrous sulphate
FID	Flame ionization detector
Fig.	Figure
FJ	Fruit Juice
FW	Fresh weight
g	Gram(s)
h	Hour(s)
h°	Hue angle
ha	Hectare(s)
H_2SO_4	Sulphuric Acid
HCl	Hydrochloric acid
HgCl	Mercury chloride
HLA	13-hydroperoxylinolenic acid
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
IAA	Indole-3-acetic acid
J	Joules
JAs	Jasmonates
kg	Kilogram(s)
KOH	Potassium hydroxide

kPa	Kilo pascals
L	Litre(s)
L*	Lightness
LSD	Least significant difference
Ltd.	Limited
LOX	Lipoxygenase
m	Meter
mm	Milli meter
M	Molar
MA	Madison
MeOH	Methanol
mg	Milligram(s)
$MgCO_3$	Magnesium carbonate
min	Minute(s)
ml	Millilitre(s)
mM	Millimolar(s)
mmol	Millimole(s)
Mt	Metric tonnes
N	Newton
n	Number of replication
NA	Not available
NAA	Naphthalene acetic acid
NaCl	Sodium chloride
NaF	Sodium fluoride
$NaHSO_3$	Sodium hydrogen sulphite
NaOH	Sodium hydroxide
NaOCl	Sodium hypochlorite

Na_2SO_4	Sodium sulphate
ng	Nanogram(s)
nl	Nanolitre(s)
nmol	Nanomole(s)
NO	Nitric oxide
NS	Not significant
NSW	New South Wales
O_2	Oxygen
ODC	Ornithine decarboxylase
OA	Oxalic acid
P	Probability
Pa	Pascals
PAL	Phenylalanine ammonia-lyase
PCIB	α（p-Chlorophenoxy)isobutyric acid
PC	Pre-climacteric
PE	Pectin esterase
PG	Polygalacturonic acid
PGRs	Plant growth regulators
pH	Symbol denoting hydrogen ion in a solution
POD	Peroxidase
ppb	Part per billion (10^{-9})
ppm	Part per million (10^{-6})
PPO	Polyphenol oxidase [catechol oxidase]
psi	Pounds per square inch
PUT	Putrescine
PRD	Partial root zone drying (PRD)

PVP	Polyvinylpyrolidone
r	Correlation coefficient
rcf	Relative centrifugation force
RH	Relative humidity
RDI	Regulated deficient irrigation
ROS	Radical oxygen species
rpm	Rounds per minute
S	South
s	Second(s)
SA	Salicylic acid
SAM	S-adenosyl methionine
dSAM	Decarboxylated-SAM
SAMDC	S-adenosyl methionine decarboxylic acid
S.E.	Standard error
sp.	Species
SPD	Spermidine
SPM	Spermine
SSC	Soluble solids concentration
Std	Standard
Treat	Treatment
TA	Titratable acidity
tan	Tangent
TAPP	Tanzania Agriculture Productivity Program
Trolox	6-hydroxy-2, 5, 7, 8-tetramethychroman-2-carboxylic acid
UK	United Kingdom
USA	United States of America
UV	Ultra-violet

VIC	Victoria
VIS	Visible
vs.	Versus
v/v	Volume by volume
WA	Western Australia
w/v	Weight by volume
w/w	Weight by weight

CHAPTER 1

General introduction

Fruits play an important role in fulfilling nutritional requirements of humans. Fruits are considered a main natural source of many nutrients, carbohydrates, vitamins, proteins, minerals, fibre, dietary polyphenols and antioxidants (Wegmans, 2009; Fu et al., 2011; Haminiuk, et al., 2012). The antioxidants inhibit the impacts of oxidative processes which cause some severe diseases in the human body, such as cancer, autoimmune diseases and multiple sclerosis (Kurosumi et al., 2007). It is interesting to note that mango fruit contain very high levels of a nutritional triterpene known as lupeol (1.80 µg g^{-1} mango pulp). Siddique and Saleem (2011) claimed that different *in vitro* and preclinical animal studies suggest that lupeol can possibly act as an anti-microbial, anti-fungal, anti-protozoal, anti-invasive, anti-proliferative, anti-angiogenic and cholesterol lowering agent.

, Fruit production estimated to be increased approximately 50% and 40% in the world and Australia respectively during the last three decades. In 2000, the total production of fruits, excluding melons, in the world was an estimated 479,172,987.00 tonnes produced on 49,602,554.00 hectares. Meanwhile, the total production of fruits, excluding melons, in Australia during 2000 was estimated to be more than 3,084,331.00 tonnes grown on 237,511.00 hectares. However, in 2013, the total production of fruits, excluding melons, in the world was an estimated 673,680,137.00 tonnes produced on 59,377,918.00 hectares. The total production of fruits, excluding melons, in Australia was estimated to be more than 3,382,166.00 tonnes produced on 275,255.00 hectares (FAOSTAT, 2015).

The postharvest losses in fresh horticultural produce are categorised into quantitative and qualitative. Postharvest losses differ greatly among horticultural crops, growing location, season and preharvest practices followed in the production phase as well as in supply chains. The postharvest losses in developing countries ranged from 30% to 44% of fresh horticultural produce in developing, and surprisingly, in developed countries as well (Kader and Siddiq, 2012; Lipinski et al., 2013; Singh, 2015). Likewise, in fruits, the postharvest losses also vary widely from 10% to 80% along the supply chain in both developed and developing countries

(FAOSTAT, 2005). The variation in postharvest losses in the Asia–Pacific region varied among different countries and ranged from 16 - 50% (Roll, 2006; Kader and Siddiq, 2012). A wide range of fruits are grown in Australia; the quantitative postharvest losses in fruits amounted to 30,902 tonnes in 2009 (FAOSTAT, 2012). The quantitative postharvest losses in horticultural crops also coupled with deterioration in quality such as texture, flavour, aroma volatile, nutritional values and cosmetic appearance (Singh, 2015). It is very difficult to quantify the monitory value of qualitative losses in horticultural produce. The reduction in quantitative and qualitative postharvest losses during supply chain management will provide high-quality fruits, contribute to increased food availability to the growing world population, reduce the area required for production, protect natural resources and decrease global warming (Singh, 2015).

Amongst various approaches to extend postharvest life and maintenance of quality of fresh horticultural produce the use of edible coatings is advocated (Mahajan et al., 2014). Edible coating materials form a thin layer on the surface of fruits and consequently creates a modified atmosphere around the produce which limits loss of water, reduces respiration and ethylene action whereby consequently retarding fruit ripening and senescence and maintaining quality (Mahajan et al., 2014). Edible coatings can be applied on fruit and vegetable surfaces in different ways such as spraying and dipping (Ghasemzadeh et al., 2008). Climacteric fruits exhibit ethylene and respiration peak during ripening whilst non-climacteric fruits do not show these ethylene and respiration peaks. Most climacteric and non-climacteric fruits are highly perishable and have short storage life at ambient temperatures. Various postharvest handling techniques such as preharvest application chemicals, postharvest heat treatment, edible coatings, cold storage, controlled atmosphere (CA) storage and modified atmosphere (MA) storage to extend the postharvest storage life of various fruits have been tested and have resulted in limited success (Baldwin et al., 1995). Various natural compounds used as a coating on fruit such as chitosan, gums, shellac, beeswax, paraffin and carnauba are safe for human health, and environmentally friendly. Many compounds have been extracted from different agricultural commodities or from waste of the food production industry to be used as edible coatings and films. The most common constituents of edible coatings are polysaccharides, proteins or lipids. Comparatively, polysaccharides have been

applied more widely compared to others to prolong the shelf-life of fruits and vegetables (Maqbool et al., 2011). Many polysaccharides (chitosan, methylcellulose or pectin) and proteins (gelatin, collagen, casein, phaseolin, zein, soy or whey proteins), or a combination of these have proven to be effective coating materials in improving glossy appearance, reducing respiration rate and water vapour loss, acting as gas exchange barriers, retarding ripening and senescence, and performing as antimicrobials consequently maintaining quality, reducing postharvest decay and extending shelf-life (Maqbool et al., 2011; Porta et al., 2013). The efficacy of edible coatings is dependent upon the kind of coating material, its concentration, storage conditions, coating layer thickness, genotype and harvest maturity (Dang et al., 2008a). Furthermore, edible coatings act as carriers when loaded with other useful compounds which modify the ethylene production and action, extend shelf life and maintain quality. For instance, application of an edible coating of chitosan incorporating thyme oil to fruit has been reported to improve fruit quality and prolong its shelf-life (Jiang et al., 2012).

Chitosan has been used successfully as a fruit coating. It is derived from chitin obtained from crustacean wastes by alkaline deacetylation. Chitosan is a linear polysaccharide containing β-(1→4)-linked 2-amino-2-deoxy-D-glucose residues. Chitosan is classified as a biodegradable polymer which is fibre-like, has a high molecular weight, is nontoxic, has antimicrobial activity, and has excellent barrier properties and low oxygen permeability. It is reported to be the second most abundant naturally occurring biopolymer after cellulose (Tamer and Copur, 2010; Fernandez-Pan and Caballero, 2011). Chitosan is not water soluble, but a viscous solution can be made in several organic acids such as acetic acid and lactic acid (Tamer and Copur, 2010). The concentrations of chitosan used for coating have ranged from 0.5 to 2 % (w/v) depending upon the kind of fruit (Zhu et al., 2008; Ali et al., 2011). Chitosan coating has prolonged shelf-life, maintained quality, minimised decay, delayed ripening, delayed colour development and loss of firmness, reduced weight loss and increased the soluble solids concentration (SSC) of fruits (Jitprakong and Changsiriporn, 2011). Therefore, due to the natural characteristics of chitosan, it has become a promising alternative postharvest treatment to protect fruit during the postharvest phase (Maqbool et al., 2010).

Chitosan films have various benefits in extending postharvest life of fruits and vegetables. Sometimes chitosan coating alone in some fruits shows certain defects, which include partial inhibition of a special microbe that leads fruit to decay, and poor coating structure to adjust the permeability of oxygen and carbon dioxide. Chitosan has an ability to be combined with other compounds to increase effectiveness such as lemon essential oil (Perdones et al., 2012), calcium chloride (El-Badawy, 2012) and oleic oil (Vargas et al., 2006). However, no research work has been reported on the effects of emulsion of chitosan loaded with salicylic acid (SA) or oxalic acid (OA) on regulation of ethylene biosynthesis, fruit softening and quality parameters such as levels of vitamin C and total antioxidants in climacteric and non-climacteric fruits.

Salicylic acid (SA) is a safe and natural endogenous phenolic compound in plants (Asghari and Aghdam, 2010). SA is a simple plant phenolic compound known to inhibit ethylene biosynthesis and impart disease resistance. Earlier, it has been reported that SA reduces ethylene biosynthesis through inhibiting lipoxygenase enzyme activity in kiwifruit (Fatemi et al., 2013). The SA increases total antioxidant capacity, vitamin C content, SSC and reduces fungal infections in strawberry (Shafiee et al., 2010). It also delays fruit softening in banana and kiwifruit during ripening (Shafiee et al., 2010). In addition, SA has been reported to decrease decay in peaches, pears, apples, nectarines and bananas, and reduce chilling injury in cucumbers and tomatoes (Mo et al., 2008). Exogenous application of SA retards ethylene biosynthesis, increases ion uptake and transport, transpiration, stomata closure and stress tolerance (Pila et al., 2010). The effects of SA on various fruit physiological processes are dependent on the concentrations applied (eg. 1 mmol L^{-1} to 4 mmol L^{-1}) and type of fruit such as strawberries, citrus, pears and apples (Asghari and Babalar, 2010; Al-Qurashi and Awad, 2012). SA has been reported to enhance flesh firmness of peaches and banana fruits during storage. Therefore, SA has a significant ability to prolong postharvest life and conserve quality during storage life of fruits (Tareen et al., 2012). No research work has been reported on the effects of emulsion of chitosan loaded with SA on regulation of ethylene biosynthesis, fruit softening, and quality parameters in climacteric fruits (eg. nectarine and plum) and non-climacteric fruit (eg. sweet orange).

Oxalic acid (OA) is an organic acid which is widespread in different living organisms such as plants, animals and fungi and plays many important physiological roles. For example, level of OA is positively associated with systemic resistance and antioxidant systems in plants (Zheng et al., 2005). In addition, it has been reported to be an anti-browning agent in litchi fruit by retarding polyphenol oxidase (PPO) activity (Zheng and Tian, 2006). Recently, OA treatment has been used for food preservation as a natural antioxidant (Zheng et al., 2007a). The exogenous application of OA (eg. 1 to 5 mM) has reported to delay senescence in many fruits (Zheng et al., 2005; Zheng and Tian, 2006; Zheng et al., 2007a). Zheng et al. (2006) reported that OA delays the loss of firmness, delays ripening and reduces ethylene production in mango fruit. The OA also lowered respiration rate and increased activities of antioxidant enzymes in peach fruit as compared with the control (Zheng et al., 2007a). In recent years, various authors have stated the beneficial effects of applying OA to delay quality deterioration and prolong the storage shelf life of many fruits, such as mango, peach, banana and sweet cherry (Cefola and Pace, 2015). There are many beneficial physiological functions of OA applications such as to reduce diseases caused by bacteria, fungi and viruses by improving the activity of defence-related enzymes. Moreover, postharvest application of OA has been found to be effective in retarding the ripening period in many climacteric commodities such as peach, mango and plum; by inhibition of ethylene production. Also, another advantage of OA postharvest application is in reducing symptoms of chilling injury (CI) in some fruits such as mango, litchi and pomegranate (Martinez-Espla et al., 2014). No research work has been reported on the effects of an emulsion of chitosan loaded with OA on regulation of ethylene production, fruit softening, and quality parameters such as levels of vitamin C and total antioxidants in the climacteric and non-climacteric fruits.

Most of the research work reported earlier focuses on the beneficial effects of chitosan, SA and OA alone in extending postharvest life and maintenance of quality of fresh horticultural produce. No research work has been reported on the effects of an emulsion of chitosan loaded with SA or OA on regulation of ethylene production, fruit softening, and quality parameters including levels of vitamin C and total antioxidants in climacteric and non-climacteric fruits. It is hypothesised that emulsion of chitosan loaded with SA or OA will be more effective compared to the

application of chitosan, SA or OA alone in prolonging postharvest life and maintaining quality of climacteric and non-climacteric fruits. Hence, the specific objectives of this research are:

1. To underpin the role of an emulsion of chitosan, SA or OA alone; and chitosan emulsion loaded with SA or OA on modulation of fruit ripening, ethylene biosynthesis, weight loss, firmness, titratable acidity (TA), SSC, SSC: TA ratio, changes in levels of sugars and organic acids, vitamin C, total antioxidants and disease incidence in the climacteric fruit of nectarine and plum at ambient temperature.
2. To investigate the effects of an emulsion of chitosan, SA or OA alone; and chitosan emulsion loaded with SA or OA on cold storage life, fruit quality including texture and levels of individual sugars and organic acids and health promoting substances in the climacteric fruits such as nectarine and plum.
3. To examine the influence of an emulsion of chitosan, SA or OA alone; and chitosan emulsion loaded with SA or OA on cold storage life, weight loss, disease incidence and fruit quality including levels of SSC, TA, firmness, texture, level of vitamin C and total antioxidants and chilling injury in the non-climacteric fruit such as late maturing ‘Midknight Valencia’ sweet orange.

CHAPTER 2

General literature review

2.1. Introduction

Rising demand for fresh horticultural produce including fruits is consistent with the increasing population of the world. Different research findings indicate that a major portion of world fresh produce (25% to 80%) becomes unsuitable for consumption due to the effect of different postharvest factors (Wills et al., 2007). Horticultural fresh produce is a perishable commodity with short shelf life which may reduce greatly in quality due to deteriorative physiological changes (Baldwin et al., 1995). The qualitative changes in harvested fresh produce occur through the changes in gaseous balance between consumption of oxygen and the production of carbon dioxide (Fig. 2.1). The gas transfer rates depend on factors such as the species, growth stage, atmospheric gaseous components (O_2, CO_2, and ethylene), temperature, and relative humidity (RH) (Kluge et al., 2002). Reducing the rate of desiccation, the physiological process of senescence and the rate of microbial growth also contributes to the extension of the postharvest life of fresh produce (Erbil and Muftugil, 1986). Use of edible coatings is one of the popular methods for extending postharvest storage life for horticultural fresh produce. The fresh produce is enrobed in the edible materials which provide a semipermeable barrier to gases and water vapour. The edible coatings also reduce the rate of respiration, production of ethylene and loss of water from fresh produce (Baldwin et al., 1995). Use of edible coatings provides an alternative to changed atmosphere storage through modification and regulation of the internal atmosphere of the fresh produce (Baldwin et al., 1996; Park, 1999). Edible coatings can carry flavours, anti-browning agents, nutrients, antimicrobial compounds, colorants and spices. This provides the potential for edible coatings to reduce the risk of pathogen growth on the food surface. Moreover, edible coatings reduce the use of synthetic packaging (Pranoto et al., 2005).

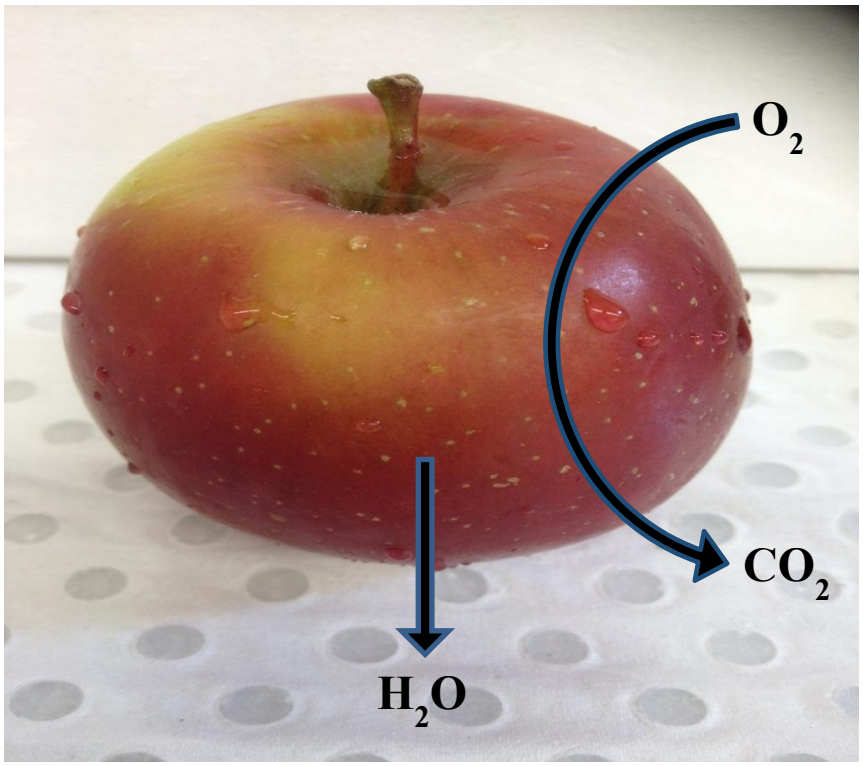


Figure 2.1. Exchange of gas and water loss from harvested fruit.

Edible coating materials can also be considered as carriers of antioxidants and preservatives (Baldwin et al., 1995). For example, in the case of citrus and peaches, edible coatings have been effectively used as carriers of antimicrobial agents such as fungicides (Brown, 1974 and 1984). Coating materials incorporated with preservatives (e.g. potassium sorbate, sorbic acid, benzoic acid, propionic acid and sodium benzoate) delay surface growth of fungi, bacteria and yeasts during storage and distribution of fresh produce (Baldwin et al., 1995). These coating materials can help hold the preservative on the fruit surface where it is required. Studies with model food systems have reported that carnauba wax holding sorbic acid is more effective than the carnauba wax alone in maintaining microbial stability in fresh produce. Antioxidative compounds added to edible films protect against oxidative rancidity, discoloration and degradation of fresh fruits. The antioxidative compounds used with coating materials have included phenolic compounds [butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), or tertiary butylated hydroxyquinone], tocopherols, or an ester such as propyl gallate. Nuts coated with pectate, pectinate, and zein coating (a maize grain protein) containing BHA, BHT and citric acid showed controlled rancidity and texture (Andres, 1984). Reduced

enzymatic browning in whole and sliced mushrooms [*Agaricus bisporus* (J.E. Lange) Imbach] was observed when they were treated with the combination of an antioxidant and a chelator (Nisperos-Carriedo et al., 1992). In spite of the wide spectrum of usefulness of edible coatings in horticultural fresh produce, there is a surge in further studies on the effect of different combinations of coating materials on the postharvest physiological performance of stone and citrus fruits during their ripening at ambient conditions or after cold storage. No research work has been reported on the effect of an emulsion of chitosan loaded with salicylic acid (SA) or oxalic acid (OA) on regulation of ethylene biosynthesis, fruit softening, and quality parameters such as levels of vitamin C and total antioxidants in nectarine, plum and citrus fruit at ambient and cold conditions.

2.2. Postharvest physiology of fresh produce

Preharvest cultural practices such as cultivar, irrigation, application of fertilisers and pesticides as well as postharvest handling of fresh produce significantly influence the quality of horticultural commodities (Dole and Wilkins, 2005). Harvested fresh products are living commodities where all the physiological and biological processes still continue; which results in deterioration of their shelf-life and quality (Sanchez-Mata et al., 2003). Heat generated through their respiration increases the endogenous temperature which speeds up metabolic processes and the qualitative deterioration of the fresh produce. Changes occurring in the postharvest period and during the process of ripening include softening, changes in flavour, aroma, colour and levels of sugars. These changes and their rate, differ according to the climatic conditions where they are produced, the cultivar, the stage of maturity, the ambient temperature and the soil (Fernando et al., 2004; Maria, 2007). Careful handling, use of anti-browning agents, ethylene inhibitors, appropriate packaging and controlled or modified atmosphere can ensure the maintenance of qualitative characteristics of harvested fresh produce for long periods (Ahvenainen, 1996; Abbott, 1999; Agar et al., 1999; Watada and Qi, 1999; Monica et al., 2003). The physiological activities occurring in harvested fresh produce are as follows:

2.2.1. Production of ethylene

After harvest, ethylene production in fruit depends on the environmental conditions to which the fruit are exposed during transport, storage and postharvest ripening

(Lelievre et al., 1997b). Ethylene production increases sharply during fruit ripening which leads to enhanced changes in texture, colour, flavour, aroma and other physiological and biochemical attributes (Burg and Burg, 1965; Dominguez and Vendrell, 1993). Produced ethylene binds to the receptors in the fruit and induces ethylene responses (Fig. 2.2) that enhance the ripening process and related events through the activation of target genes involved in fruit softening, sugar and acid metabolism (Solano et al., 1998; Adams-Phillips et al., 2004). The cumulative action of these genes results in the development of pigments, degradation of chlorophyll and cell walls leading to softening, conversion of starch to sugar, accumulation of secondary metabolites and production of aroma volatiles (Giovannoni, 2004; Stepanova and Alonso, (2005).

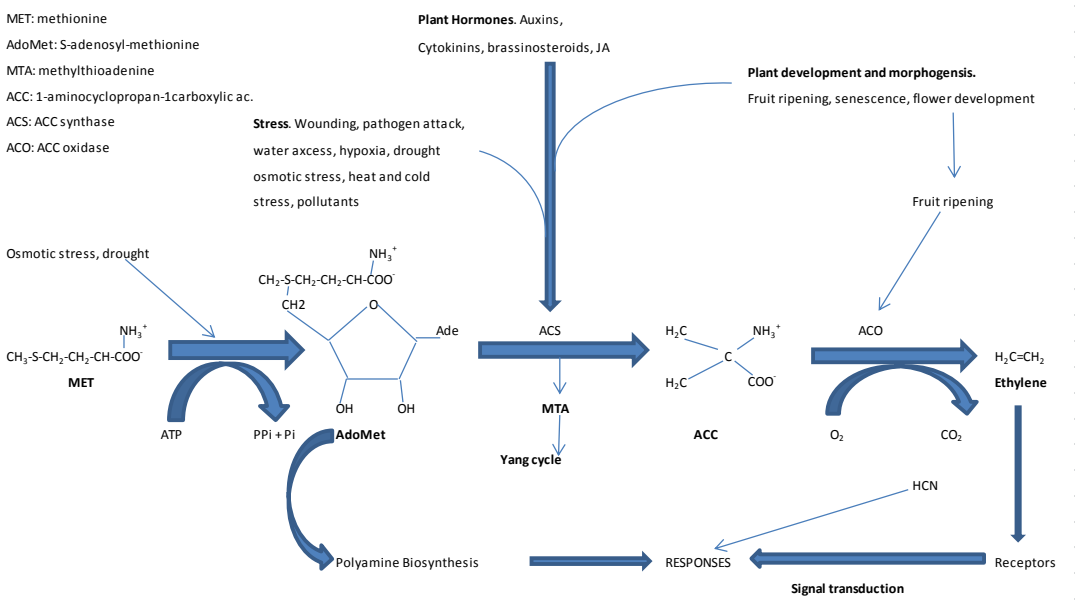


Figure 2.2. Factors related to ethylene biosynthetic and signalling showing the different enzymes involved in the process. The schematic figure showing the role of stress conditions, plant hormones and developmental stages on ACS, ACO and AdoMet. Polyamine biosynthesis starts from AdoMet which may interact with ethylene biosynthesis and plant responses to stress (Argueso et al., 2007).

Ethylene is a simple gaseous olefin and only a trace amount is needed to initiate ripening and senescence in climacteric fruits (Lelievre et al., 1997a; Bleecker and Kende, 2000; Pech et al., 2002; Nath et al., 2006; Chaves and De Mello-Farias, 2006; Tharanathan et al., 2006). Ethylene also influences the biosynthesis of aroma

volatiles in ripening of mango fruit (Lalel et al., 2003e). Higher levels of total aroma volatiles, monoterpenes, sesquiterpenes, alcohols, aldehydes, total esters and tetradecane in the mango fruit treated with the exogenous application of an ethylene releasing chemical such as ethephon (500 to 2000 mg L^{-1}) were also reported by Lalel et al. (2003e). Identification of tomato mutants differing in ethylene production and/or sensitivity to ethylene also indicated the relationship between ethylene and ripening of fruit (Gray et al., 1994; Barry et al., 2005; Barry and Giovannoni, 2006). Ethylene produced in lower concentrations triggers the entire array of changes occurring during ripening of climacteric fruit. Only 0.01µL L^{-1} and 0.05-0.25 µL L^{-1} ethylene is sufficient to trigger the ripening process in mango and banana respectively (Johnson et al., 1997).

2.2.2. Respiration

Stored carbohydrates in harvested fresh produce are broken down through the respiration process to produce the necessary energy for maintaining cellular processes and keeping the fresh produce alive. The respiration process includes consumption of oxygen with release of CO_2, water and energy (Fig. 2.1) which ultimately affects the flavour, colour, sweetness and content of water and nutrients in the fresh produce (Kays and Paull, 2004). The rate of respiration in the fresh produce depends on the cultivar (Araiza et al., 2005), harvest maturity stage (Mohammed and Brecht, 2002), pre- and postharvest environmental factors (Chonhenchob and Singh, 2004), temperature during storage (Nakamura et al., 2003), atmospheric composition (Nakamura et al., 2004), level of exposure to ethylene (Lalel et al., 2003d; Nair and Singh, 2003; Montalvo et al., 2007), and level of mechanical injury and decay (Mohammed and Brecht, 2002) (Fig. 2.3).

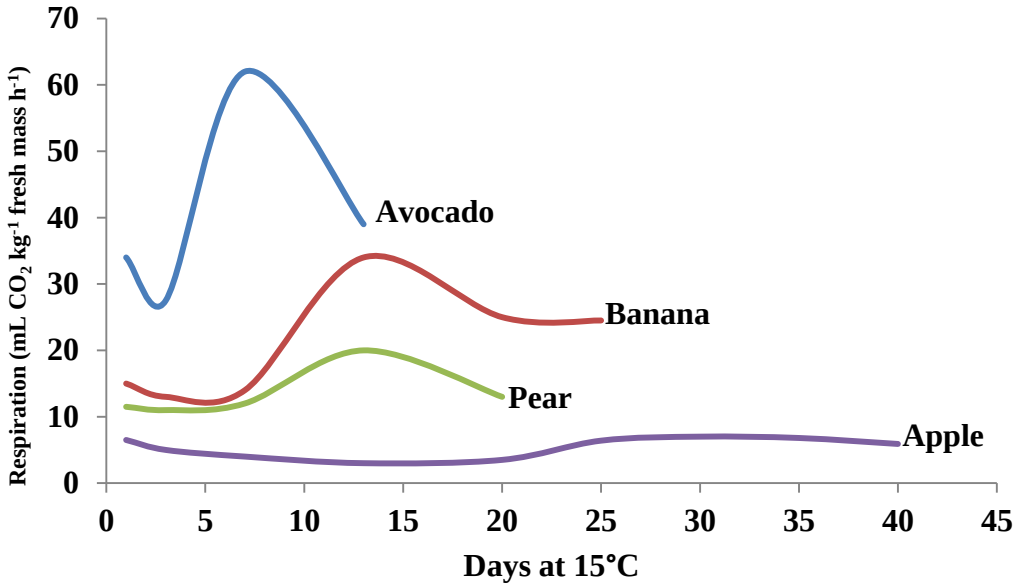


Figure 2.3. Variations in respiratory output of CO_2 in different climacteric fruits during ripening at 15°C (Baile, 1950).

2.3. Weight loss

Water content is reduced in fruit by release to the surrounding area as water vapour through transpiration. This involves the movement of water from fruit cells (100% RH in fruit intercellular spaces or internal atmosphere) to the surrounding atmosphere in storage environments which contain reduced moisture in the air (reduced % RH). For this reason a fresh crop is mostly stored under specific conditions of high RH (90%–98%) to reduce water loss, weight loss, and shrivelling. Edible coatings are used to help delay this movement of water vapour but they become more permeable to water vapour and gases under conditions of high RH (Baldwin, 2007). Fruit weight loss is mostly related to respiration and moisture evaporation through the skin. The rate of water loss is based on the level of water pressure between the fruit tissue and the surrounding area, and the temperature of storage. Edible coatings act as barriers, thereby controlling water movement and protecting the skin of fruit from mechanical injuries, as well as closing small wounds and hence retarding dehydration. For example, at the end of storage, uncoated strawberries exhibited 28.7% loss in weight, while the weight losses of those coated with 1.0% and 1.5% chitosan were 19.6% and 14.2%, respectively. Similarly, in the case of grapes, weight loss happened mainly during the first three days of storage and was clearer for the control samples and those coated with a pure chitosan coating

than for those with chitosan coatings loaded with bergamot oil which demonstrated the lowest weight losses (Shiekh et al., 2013).

2.4. Fruit softening

Fruit firmness is one of the key parameters in determining consumer acceptance and depends on cultivar and changes in cell walls and pectic materials in the middle lamella (Selvaraj and Kumar, 1994). The pectins present in the inner mesocarp tissue are more soluble than the outer mesocarp (Mitcham and McDonald, 1992; Lazan et al., 1993) and higher solubility of cell wall pectins promotes softening in fruit (Roe and Bruemmer, 1981; Tandon and Kalra, 1984; Lazan et al., 1986; Nasrijal, 1993). Depolymerisation of pectin in fruit is enhanced by cell wall hydrolases which begins in the early ripening stage of the fruit and continues throughout the ripening period (Prasanna et al., 2003 and 2005; Ali et al., 2004; Chourasia et al., 2006; Chourasia et al., 2008). Depolymerisation of matrix glycans reduces the rigidity of cell walls and induces fruit softening (Negi and Handa, 2008). The rate of depolymerisation may be very slow (e.g. apple, strawberry, banana and bell pepper); or progressive, which begins slowly and increases substantially in late ripening (e.g. kiwifruit, tomato, avocado and papaya); or abrupt, absent in early ripening but occurring rapidly in late ripening (e.g. melon and melting flesh peach) (Negi and Handa, 2008).

2.5. Changes in fruit colour

Change in fruit skin colour is an important signal of harvest maturity and it occurs due to accumulation of anthocyanins in fruit skin (Gonzalez-Aguilar et al., 2001; Cocozza et al., 2004; Mahayothee et al., 2004; Jha et al., 2007). The changes in the skin colour are the result of transformation of chloroplasts containing green coloured chlorophyll to chromoplasts containing yellow colour (Xanthophyll) or orange colour (β-Carotene) (John et al., 1970; Lakshminarayana, 1980; Parikh et al., 1990; Lizada, 1993). The yellow skin of fruit at ripe stage contains mostly carotenoids and xanthophylls and the anthocyanin paenoidin-3-galactoside dominates in the fruit skin with reddish colour (Proctor and Creasy, 1969). Substantial decrease in the concentration of chlorophyll occurs in 'Keitt' mangoes while the concentration of carotenoids rises and anthocyanin declines gradually in 'Tommy Atkins' during fruit ripening leading to colour change from green to yellow (Medlicott et al., 1986). However, change in peel colour is not an accurate indicator of maturity index for

those fruit where the fruit softening occurs before the changes in skin colour (Mitcham and McDonald, 1992). The development of fruit colour depends on harvesting period (Shafiq et al., 2011), availability of light through the tree canopy (Layne et al., 2002); orchard temperature before harvesting (Iglesias et al., 2002); treatment with chemicals such as methyl jasmonate (Shafiq et al., 2012), paclobutrazol (Antognozzi and Romani, 1989), ethylene (Saure, 1990), aminoethoxyvinylglycine and ethephon (Whale and Singh, 2007; Whale et al., 2008) and fruit bagging (Fan and Mattheis, 1998). Reduction in the level of ethylene production in apple delays the development of colour through inhibiting the biosynthesis of anthocyanin (Lancaster, 1992). Whale et al. (2008) observed that the treatment with ethephon degrades chlorophyll and improved red colour on the 'Cripps Pink' apple fruit skin and application of ethylene biosynthesis inhibitor such as AVG retarded the degradation of chlorophyll and development of red colour on the fruit surface. Yamauchi et al. (1997) also observed increased chlorophyll degradation and improved orange colour development in 'Wase Satsuma' mandarin with ethylene treatment. Clayton et al. (2000) reported reduced loss of chlorophyll and retarded colour development in 'Bartlett' pears with AVG treatment. Excessive nitrogen application could be the main reason for the green colour to persist in the 'BC-2 Fuji' apple fruit at harvest time. Hence, the internal maturity of the fruit is determined by estimating ethylene emission and respiration process, which are considered as indicator to determine appropriate harvest time (Fallahi et al., 2001).

2.6. Changes in aroma

Different pre- and postharvest factors such as cultivar, harvest maturity, ripening stage, storage conditions and postharvest treatments with growth regulators such as ethylene and jasmonates affect the production of aroma volatiles (Lalel et al., 2001; Lalel, 2002; Lalel et al., 2003a; Lalel et al., 2003b; Lalel et al., 2003c; Lalel et al., 2003d; Lalel et al., 2003e; Lalel et al., 2003f; Nair et al., 2003; Lalel et al., 2004a; Lalel et al., 2004b; Singh et al., 2004; Lalel and Singh, 2006). Other important factors including rootstock (Dang, 2007), application of polyamines, hot water dip (Dea et al., 2010), fungicide treatments (Dang et al., 2008b) and edible coatings (Dang et al., 2008a) have also been observed to influence aroma volatile production in ripe fruit. Terpenes are the maximum abundant combinations among the aroma volatiles in mango which also contains esters, ketones and lactones (Lalel et al.,

2003a). Volatile compounds are mostly hydrocarbons and esters; accounting for about 59% and 20% respectively. The production of terpenes and esters are positively correlated with the biosynthesis of ethylene and fatty acids respectively (Lalel et al., 2003a). Lalel et al. (2004b) observed that most of the fatty acids in mango pulp increased with the increase in ripening temperature and fruit ripened at 25°C exhibited significantly higher concentrations of individual fatty acids than fruit ripened at 15°C, 20°C, 30°C and 35°C. A significant positive correlation between carotenoids and norisoprenoids was also reported by Lalel et al. (2004b). Maturity status also affects the amount of aroma volatiles in fruit. Lalel et al. (2003d) observed that the pulp of ripe fruit harvested at the sprung green stage contain higher amounts of aroma volatiles, monoterpenes, sesquiterpenes and aromatics.

2.7. Changes in soluble sugars

The concentrations of soluble sugars increase during the ripening of mango fruit resulting in increased sweetness (Ito et al., 1997). The effect of different pre- and postharvest factors on the content of soluble sugar in plum (Taylor et al., 1995; Singh and Singh, 2008; Usenik et al., 2008b), sweet cherry (Usenik et al., 2008a), peach (Chapman and Horvat, 1990; Robertson et al., 1990; Chapman et al., 1991; Wu et al., 2005), apple (Ackermann et al., 1992; Chardonnet et al., 2003), pear (Itai and Tanahashi, 2008) and loquat (Ding et al., 1998) have been reported. Ripe mango fruit contains 10 – 20% total sugars (Litz, 2009). Accumulation of sugars depends on the level of starch content in the fruit which is hydrolysed to sugars (Kumar et al., 1994; Selvaraj et al., 1989; Singh et al., 2009). In 'Kensington Pride' mango the SSC increases from 6.2% to 14.0% (O'Hare, 1995), in 'Keitt' mango from 4.9% to 11.6% (Medlicott and Thompson, 1986) and in 'Alphonso' mangoes from 7.0% to 15.0% (Thomas, 1975). Ito et al. (1997) noted a higher level of starch content (14%) in most of the mango cultivars at the green stage than ripe stage (0.3%). An increase in the level of glucose, fructose and sucrose during fruit ripening in mango has also been reported by Krishnamurthy et al. (1971), Lakshminarayana (1975) and Shashirekha and Patwardhan (1976). Higher accumulation of sucrose during fruit ripening occurs through increased starch hydrolysis by α and β amylase (Mattoo and Modi, 1969; Fuchs et al., 1980; Tandon and Kalra, 1984). During the rapid accumulation of sucrose in fruit, Castrillo et al. (1992) recorded ten times the activity of sucrose synthase (SS) enzyme.

2.8. Changes in organic acids

The concentration of organic acids determines the flavour in ripe fruit (Guerra and Casquero, 2008). The citric and malic acids are predominant acids in most mature fruit and the concentration of organic acid decreases substantially from maturation to ripening stages (Singh et al., 2009; Zahara and Singh, 2011c). Other organic acids e.g. fumaric, shikimic, tartaric and succinic acid are present in a low concentration in mango fruit (Shashirekha and Patwardhan, 1976; Sarker and Muhsi, 1981; Medlicott and Thompson, 1986; Kumar et al., 1993; Singh and Singh, 2012). The concentration of citric acid increases steadily in 'Irwin' mangoes which reaches a higher level at the initial stage of endocarp-hardening and declines in the matured fruit during ripening (Ito et al., 1997). Lizada (1993) also observed a reduced level of citric and succinic acids during mango fruit ripening. The decrease in the level of acidity in ripe fruit is due to the losses in citric and malic acids (Medlicott and Thompson, 1986). The activity of malic dehydrogenase and succinic dehydrogenase increased during the onset of ripening; whereas, activity of citrate synthase (CIS) rises several-fold during maturation in 'Alfonso' mangoes (Baqui et al., 1974). Dubery et al. (1984) noted the higher activity of malic enzyme just after the climacteric peak and the level of malic acid declined in the post-climacteric stage of the fruit ripening.

2.9. Maintaining postharvest quality of fresh produce

Controlling the concentrations of ethylene, oxygen (O_2) and carbon dioxide (CO_2) in the storage atmosphere, temperature and humidity during storage can ensure better quality in harvested fruit (Saltveit, 1999). Among the postharvest practices, use of edible coatings has been recognized as a potential measure to maintain the postharvest characteristics of the fresh produce. The use of edible coatings and their role in maintaining postharvest qualities and extending shelf life of fresh produce will now be described.

2.10. Use of edible coatings

Edible coatings are thin layers of edible materials used in addition to, or as a replacement for, the natural protective waxy coatings on fresh produce to create a modified atmosphere by providing a barrier to moisture, oxygen and solute movement (Smith et al., 1987; Nisperos-Carriedo et al., 1992; Guilbert et al., 1996; Lerdthanangkul and Krochta, 1996; Avena-Bustillos et al., 1997; McHugh and

Senesi, 2000). Storage life of fresh produce can be extended by using an ideal coating which will not cause anaerobic respiration (leading to production of off flavours) and will reduce decay without deteriorating product quality (McHugh and Senesi, 2000). The effect of edible coatings on fresh produce depends on thickness, temperature, alkalinity and type of coating, and the variety and condition of the fresh produce (Park et al., 1994 a and b). A combination of beeswax and sodium caseinate was found to have lower water vapour permeability than stearic acid or acetylated monoglyceride (Avena-Bustillos and Krochta, 1993). Wong et al. (1994) reported that a double layer coating of polysaccharide and lipid increased water vapour resistance in cut apple by 92%, reduced respiration by 70%, and decreased ethylene production by 90%. Edible coatings have been applied for centuries on fruits and vegetables (Hardenburg, 1967). To retard the transpiration loss in citrus fruit the Chinese started to use wax coating in the early 12^{th} century and recorded extended shelf life of the treated fruit. Later in the 1930's paraffin waxes became commercially available and started to be used as edible coatings for apples and pears (Krochta and Mulder-Johnston, 1997). A 2.5 mm thick film layer of oils, waxes, or cellulose has been found to prevent spoilage and retain the quality of fresh produce (Nisperos-Carriedo et al., 1990; Baldwin et al., 1995). Lowings and Cutts (1982) used a mixture of sucrose fatty acid esters (SFAE), sodiumcarboxymethyl cellulose, and mono- and di-glycerides to retard the ripening of fruits. Tomato fruit coated with zein (a maize grain protein) showed delayed colour change, weight loss and maintained firmness during storage (Park et al., 1994 a and b).

2.11. Materials used as edible coatings

Edible coatings generally constitute one or more of four main kinds of materials namely proteins, polysaccharides, lipids and resins (Baldwin et al., 1995). A plasticizing compound (e.g. oils, waxes, and polyhydric alcohols) is added to improve flexibility and elasticity of coating substances (Chuah et al., 1983; Andres, 1984; Gennadios and Weller, 1990). Release agents and lubricants are added to prevent coated fresh produce from sticking among coated fruit. Lipid-based coatings are prepared from oils and waxes (Baldwin et al., 1995). The wax materials include paraffin wax, carnauba wax, beeswax and candelilla wax. Stearic acid, lauric acid, vegetable oil, mineral oil, acetylated monoglycerides, or sucrose esters of fatty acids are considered are also oil components used as part of coating materials (Hagenmaier

and Shaw, 1990). These materials have been used extensively on horticultural fresh and cut produce (Pennisi, 1992; Avena-Bustillos et al., 1994; Wong et al., 1994a).

Different polysaccharides such as alginates, pectin, cellulose, chitosan, starch, carrageenan and gums are generally good gas barriers and adhere well to the surfaces of fresh produce (Kester and Fennema, 1988). Chitosan is a deacetylated form of chitin which inhibits the growth of infectious fungi (Allan and Hadwiger, 1979; Stossel and Leuba, 1984; Hirano and Nagao, 1989) and has shown promising performance in extending shelf life of fresh horticultural produce (Pennisi, 1992). Protein compounds used as edible coatings include casein, gelatin, soy, zein and egg albumen. They are good film-formers and adhere to hydrophilic surfaces, however, they are less effective in resisting water vapour diffusion compared to other types of films (Rendell-Dunn, 1990; Gennadios and Weller, 1990). Some casein-containing coatings and soy proteins have been observed to improve the quality of horticultural fresh produce (Kinzel, 1992; Avena-Bustillos et al., 1993, 1994; Wong et al., 1994b). Different combinations of different types of coating materials have been used to form multiple layers to improve gas exchange, adherence to product, and moisture vapor permeability of the coatings (Baldwin et al., 1995). Wong et al. (1992) reported that a chitosan–lauric acid film forms a unique film structure and improves the water resistance property of the coating. Similar observations were reported by Kester and Fennema (1989) in a study on lipid films such as beeswax, polyvinyl chloride and polystyrene resistance to water vapour transmission and concluded that these combinations of films are promising for coating fresh horticultural produce. Further details on the reported use of some of the important coating materials will now be presented.

2.11.1. Chitosan

Chitosan is a natural biopolymer derived from chitin and can be extracted from many natural sources such as exoskeletons of crustaceans, molluscs, fungi and insects. The backbone structure of cellulose, chitin, and chitosan is very similar (Fig. 2.4) and they differ only in the functional group at C-2 position. Chitin consists of 2-acetamido-2-deoxy-β-D-glucose linked through β (1 → 4) bonds in which the hydroxyl group at C-2 position in the glucose residues cellulose has been replaced with the acetamido group (Luo and Wang, 2013). Chitosan is derived from chitin by

the N-deacetylation process which places the amino group at C-2 position on its backbone.

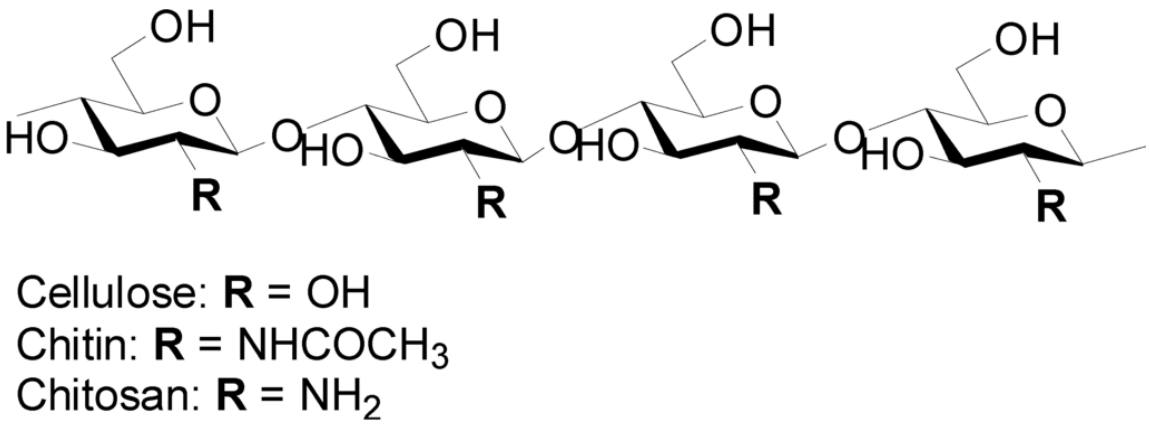


Figure 2.4. Chemical structures of cellulose, chitin and chitosan (Ifuku, 2014).

Chitosan is a versatile biopolymer that can be formulated into films, gels, beads, and nano/micro-particles, and can be used in several applications including use in food, drugs, and cosmetics. In addition, chitosan is well-known for its low toxicity, biodegradability and biocompatibility. Chitosan's hydroxyl and amino groups on its backbone allow its further modification to improve physicochemical properties for its easy applicability in different situations (Mourya and Inamdar, 2008). Due to this flexibility, chitosan has received increasing attention and extended applications in all aspects of science. Chitosan is considered as GRAS (Generally Recognized as Safe) by the Food and Drug Administration (FDA) in the USA for use with food commodities (Yen et al., 2008). Chitosan is now being used widely in agriculture and the food industry due to its antimicrobial and structural properties that allow its use as an edible coating as a natural antioxidant (Ai et al., 2008 and 2012). Bautista-Banos et al. (2006) reported that chitosan controls pathogenic microorganisms in different types of fruits. In addition, chitosan has been stated to control postharvest diseases of citrus fruit (Zhang et al., 20011). Chitosan has been used to control pre- and postharvest diseases of horticultural fresh produce, however some detrimental effects on quality of fruits were treated with chitosan (>1.5%) (Zahid et al, 2012a; Zahid et al, 2012b). Zahid et al. (2012a) also observed that the nanoemulsion form of chitosan (particle size < 1000 nm) is cheaper and more effective than the

conventional emulsion of chitosan (particle size > 1000 nm) to work as a biofungicide for controlling anthracnose in fresh fruits. Use of chitosan as an edible coating has great potential to control postharvest diseases of fruits and has potential to reduce the use of fungicides which may have environmental benefits (Zeng et al., 2010). No research work has been reported on the effect of an emulsion of chitosan loaded with SA or OA on regulation of ethylene biosynthesis, fruit softening, reduction of weight loss and disease incidence and quality parameters such as levels of vitamin C and total antioxidants in nectarine, plum and citrus fruit at ambient and cold storage conditions.

2.11.2. Salicylic acid (SA)

Salicylic acid is an endogenous phenolic growth regulator (Karlidag et al., 2009) which has been extensively used for quality improvement of fresh produce (Peng and Jiang, 2006). It influences the physiological or biochemical processes such as enzymes activity, membrane permeability, nutrient uptake, growth and development in plants (Arberg, 1981). SA is a natural and safe phenolic compound (Fig. 2.5) with potential to control post-harvest losses of fresh produce and has been stated to control a number of processes in plants including ethylene production, seed germination and sex polarization (Raskin, 1992; Zhang et al., 2003). Treatment with SA results in suppressed ethylene production, lower rate of respiration, and induction of resistance to disease, oxidative stresses and chilling injury in fresh produce. SA treatment also delays the ripening and senescence process, prevents the activity of cell wall degrading enzymes such as chitinases, glucanases and pectinases and thus maintains firmness of fresh produce (Romani et al., 1989; Zhang et al., 2003). SA has been reported to suppress ethylene production consequently inhibiting lipoxygenase (LOX) activity resulting in retardation of kiwifruit ripening (Xu et al., 2000). Preharvest treatment with SA on a commercial scale can induce resistance to postharvest diseases in fruits and vegetables. SA also improves the influences of other postharvest treatments such as heat treatments and biocontrol agents (antagonist yeasts and *R.glutinis*) which results in better control of post-harvest losses (Asghari and Aghdam, 2010). SA shows antifungal effects on some plants and harvested fruits (Huang et al., 2000; Amborabe et al., 2002). Lu and Chen (2005) have observed the inhibitory action of SA on *Botrytis* rot in lily leaves. Rock melons

and Hami melons treated with Asilbenzolar-S-methyl (a synthetic analogue of SA) showed resistance to postharvest diseases (Huang et al., 2000).

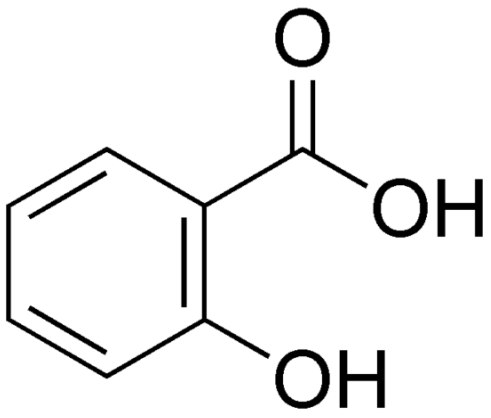


Figure 2.5. Salicylic acid

Fruit dipping in SA solution (0.01mM to 1.0mM) significantly decreases the deterioration of qualitative properties such as chilling injury in peaches (Wang et al., 2006), tomato (Ding et al., 2001), sweet peppers (Fung et al., 2004), and loquat fruits (Cai et al., 2005). SA maintains the flesh firmness in harvested peaches (Yan et al., 1998; Li and Han, 1999; Wang et al., 2006) and banana fruits during storage and ripening (Srivastava and Dwivedi, 2000). SA also mitigates the deleterious effects of chilling (Korkmaz et al., 2007; Horvath et al., 2007), high temperature and drought (Senaratna et al., 2000), and salinity (Yildirim et al., 2008) in plants. It is a compound with very low toxicity LD50 (rat) 891 $mgkg^{-1}$. SA activates the expression of several defence-related genes (Lu et al., 2003). Treatment with SA (1.0 mM) reduces physiological decay in banana, nectarine, peach, apple and pear (Yan et al., 1998; Zhang et al., 2003) and decreases the rate of chilling injury in tomato and cucumber when stored at low temperature (Han et al., 2002). Moreover, SA prevents cardiovascular diseases in humans (Deng et al., 2001) and is suitable for use with harvested fruits as a food additive (Mo et al., 2008). Several studies indicate beneficial influences of SA treatment on extending storage life of fruits. For example, during ripening, endogenous levels of SA decrease coupled with accelerated softening, and exogenous application of acetylsalicylic acid (a derivative

of SA) resulted in higher endogenous levels of SA consequently inhibiting ethylene production leading to delayed fruit softening in kiwifruit (Zhang et al, 2003). Application of SA at either pre-harvest or postharvest stage reduced fungal decay in sweet cherry (Yao and Tian, 2005; Xu and Tian, 2008), strawberry (Babalar et al, 2007; Shafiee et al, 2010) and peach fruits (Wang et al, 2006) by inducing the defence resistance systems and stimulating the activity of antioxidant enzymes (Khademi and Ershadi, 2013). Preharvest application of SA induces resistance to pathogens in pear (Jiankang et al., 2006) and decreased disease development in cherry (Yao and Tian, 2005). SA (2 mM) effectively increases total antioxidants, ascorbic acid content, soluble solids concentration and reduce fungal contaminations in strawberry fruit (Asghari, 2006; Shafiee et al., 2010). Mango fruit treated with SA show lower level of chilling injury than untreated fruit (Liu et al., 2007; Al-Qurashi and Awad, 2012). Being a natural inducer of disease resistance, SA shows antifungal activity against some pathogens of mango, citrus and pear (Zainuri et al., 2001; Shaat and Galal 2004; Cao et al., 2006; Iqbal et al., 2012). For prolonged postharvest life in oranges, with maintained nutritional quality Huang et al. (2008) suggested that they could be pre-treated with SA and stored at low temperature. Application of SA delays ripening, increases disease resistance and maintains quality of banana, mango, sweet cherry and kiwifruit; reduces chilling injury in pomegranate, peach, tomato, cut rose flower and sweet peppers; and reduces lipid peroxidation in navel orange (Kant et al., 2013). SA inhibits ethylene biosynthesis and delays the senescence process in fresh produce (Ozeker, 2005) by inhibiting the conversion of ACC into ethylene (Leslie and Romani, 1988) and suppressing ACC oxidase activity (Fan et al., 1996). No research work has been reported on the effect of SA loaded with an emulsion of chitosan on suppression of ethylene production, maintaining of fruit softening, and quality parameters such as levels of individual sugars and organic acids, vitamin C and total antioxidants in nectarine, plum and citrus fruit at ambient and cold conditions.

2.11.3. Oxalic acid (OA)

Oxalic acid exists in living organisms (Fig. 2.6) as an organic acid (Libert and Franceschi, 1987; Shimada et al., 1997). It can be obtained from vegetables and has been applied as an anti-browning agent on apple slices (Son et al., 2001). In rhubarb, beetroot and spinach it is present at a level of 100-780 mg $100g^{-1}$ fresh weight

(Hodgkinson, 1977). Dipping in OA solution is currently being used as an anti-browning agent on harvested vegetables (Castaner et al., 1997), spinach (Sato, 1980), sunflower (Marciano et al., 1983) apple slices (Ferrar and Walker, 1993; Son et al., 2001), litchi fruit (Zheng and Tian, 2006) and banana slices (Yoruk et al., 2002). Its application decreases PPO activity (Yoruk, et al., 2002) which is responsible for browning in fresh produce. OA induces systemic resistance to fungi, bacteria and viruses pathogens and enhances the antioxidant systems in plant organs which have led to interest in its potential to be used as a postharvest treatment to fruit (Mucharroman and Kuc, 1991; Zhang et al., 1998; Zheng et al., 1999; Malencic et al., 2004; Tian et al., 2006). OA dip treatment (5 mmol) significantly inhibited blue mould rot (caused by *Penicillium expansum*) in jujube fruit (Wang et al., 2009). OA (5.0 mM) dip application also delays ripening of mango fruit and reduces decay by minimising CI during storage (Zheng et al., 2005). Peach fruit stored at ambient conditions showed suppressed rate of respiration, increased activity of antioxidant enzymes, retained membrane integrity and delayed ripening processes when they were treated by dipping in 1 and 5.0 mM OA solution (Zheng et al., 2007a; Tareen, 2011). No research work has been reported on the effect of OA loaded with an emulsion of chitosan on reduction of ethylene production, maintaining fruit softening, reduction of weight loss and disease incidence and quality parameters such as levels of vitamin C and total antioxidants in nectarine, plum and citrus fruit at ambient and cold conditions.

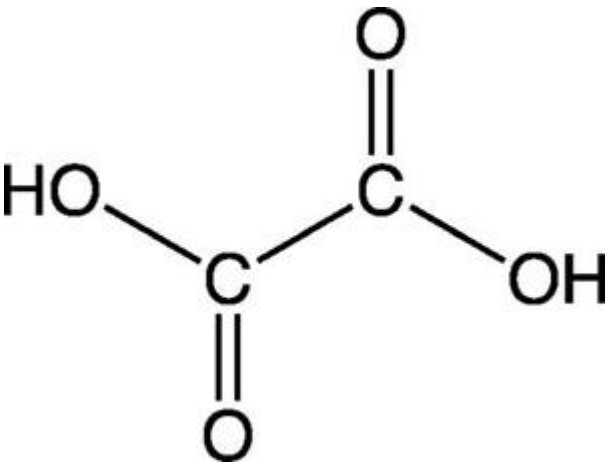


Figure 2.6. Oxalic acid

2.12. Effects of edible coatings

The principal function of an ideal edible coating is to retard the loss of moisture and desirable flavour volatiles from the coated fresh produce by restricting the exchange of CO_2 and O_2, thus creating a modified atmosphere (MA) (relatively higher CO_2 and lower O_2). This MA slows down the production of ethylene, the rate of respiration and inhibits ethylene action. Edible coatings create a semi-permeable membrane to reduce the rate of respiration, ethylene production, and moisture loss during postharvest handling and processing (Nisperos-Carriedo et al., 1990). Horticultural fresh produce continue to respire by using their endogenous oxygen and while they are coated the used oxygen cannot be replaced from the atmosphere which leads to an accumulation of CO_2 within the produce. As a result, the fresh produce shifts to a partially anaerobic respiration that requires less oxygen (1–3%) which ultimately inhibits production of ethylene and minimizes physiological loss of water (Park et al., 1994a and b; Guilbert et al., 1996). Thus, the fresh produce remains firm and fresh for longer periods with various quality parameters maintained such as firmness, weight loss and vitamin C content. The type and amount of coating influences the extent of changes in the internal atmosphere (oxygen and carbon dioxide) and the level of suppression of weight loss (McHugh and Senesi, 2000).

2.13. Factors affecting the performance of edible coatings

The performance of edible coatings depends on their molecular structure rather than molecular size and chemical constitution. The properties and performance of coatings are dependent on the ambient temperature and relative humidity conditions. For instance, the relative humidity (RH) during storage can affect the gas barrier properties of edible coatings (Baldwin et al., 1995). The rate of respiration in edible coated fresh produce increases significantly with an increase in storage temperature. Even though an appropriate MA is created by the edible coating, high storage temperatures for an extended period can cause anaerobic conditions leading to off flavour in the produce. During low RH storage, the coating materials may dry out resulting in moisture loss from the coated fruit tissue which is in contrast to the purpose of coating fresh produce (Baldwin et al., 1995). Various factors such as type of coating, size of coating particles, thickness of coating on the fruit surface, type of fruit, and harvest maturity of fruit influence the efficacy of coating in extending postharvest life and maintaining fruit quality.

2.14. Conclusion

Application of edible coatings for fresh fruit is generally still at the 'in trial' stage. The above review is a summary of what has been published in the literature regarding use of edible coating. It is evident that the research on edible coatings is mostly confined to the application of coating material alone. Further research is warranted to explore the effects of a combination of polysaccharide (e.g. chitosan) and natural compunds such as SA, OA and different types of essential oils on fresh produce. The reviewed literature also reveals that there is no information available on the effect of chitosan emulsion loaded with SA or OA on climacteric (e.g. nectarine, plum) and non-climacteric (e.g. sweet orange) fruit ripening, extending storage life and maintaining fruit quality. The critical analsysis of the literature suggests investigation is needed of the effects of the coating treatments of chitosan emulsion, SA and OA alone or chitosan emulsion loaded with SA or OA on extending storage life at ambient and low temperature and maintaining fruit quality in nectarine, plum and sweet orange. The current study was designed on the basis of the reviewed literature to fulfil these objectives.

CHAPTER 3

General materials and methods

A number of experiments were conducted to evaluate the effects of edible coating treatments with emulsions of chitosan, salicylic acid (SA) or oxalic acid (OA) alone; and chitosan emulsion loaded with SA or OA on postharvest physiological and physico-chemical properties of some selected climacteric (nectarine and plum) and non-climacteric (sweet orange) fruit. The materials used and the methods followed in these experiments are presented in this chapter.

3.1. Fruits

Fruits used in the study included mature, visually disease free and uniform sized fruit of 'Honey Fire' and 'Bright Pearl' nectarines; 'Tegan Blue' and 'Angelino' Japanese plums; and 'Midknight Valencia' sweet orange. The 'Honey Fire' and 'Bright Pearl' nectarines were sourced from Casuarina Valley Orchard, Karragullen, Perth Hills (31° 57′S/ 115° 50′E) Western Australia; 'Tegan Blue' and 'Angelino' Plum from Balingup (33° 47'S/ 115° 59'E) Western Australia and 'Midknight Valencia' sweet orange from Moora Citrus Orchard (30° 35'S/ 115° 55'E), Dandaragan, Western Australia (Fig. 3.1). The fruit were harvested in the early morning of the day of collection and transported to the Horticulture Research Laboratory, Technology Park, Curtin University, WA, by using a temperature controlled (15 ± 1°C) vehicle immediately after sorting out the hard mature and disease free fruit. Proper care and precautions were taken during transportation and after reaching the destination to prevent any loss of quality of the collected fruit.

3.2. Experimental conditions

Treated and untreated fruits were allowed to ripen at ambient conditions (Temperature 20 ± 2°C and R.H. 70 ± 5%). In some cases, treated and untreated fruit were also kept in cold storage (0 ± 1°C and R.H. 95 ± 3% for nectarine and plum or 3°C and 7°C and R.H. 95 ± 3% for sweet orange) as per the design and set up of the experiment.

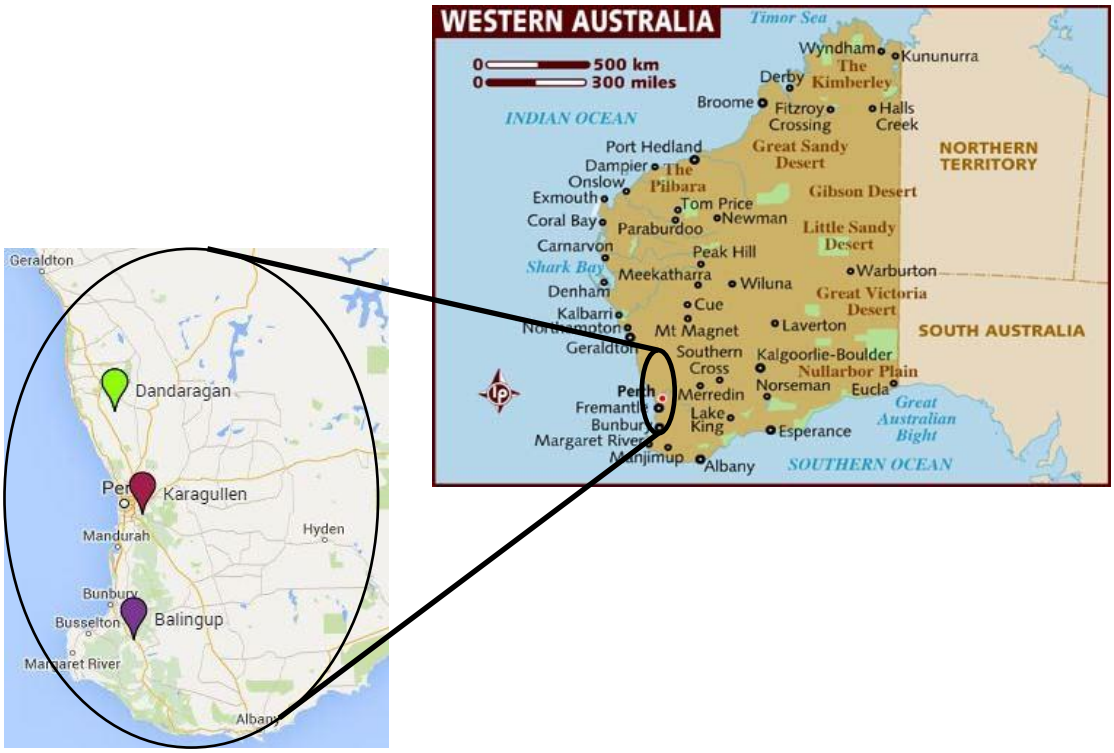


Figure 3.1. Locations for collection of experimental fruit in WA including, Karagullen, Balingup and Dandaragan from where mature nectarine and plum and ripe sweet orange fruits were collected respectively (ATTN, 2014).

3.3. Experimental method

3.3.1. Design of experiments

The experiments were conducted by following two or one factor factorial completely randomized design (CRD) with four replications and 10 fruit in each replication. Separate experiments were conducted with the selected kinds of fruit and different experimental conditions. The experiments were conducted to evaluate the effects of emulsions of chitosan, salicylic acid (SA) or oxalic acid (OA) alone; and chitosan emulsion loaded with SA or OA on ripening and quality of the fruit kept at ambient conditions or cold storage (0-1°C) or (3°C and 7°C).

3.3.2. Preparation and application of the coating materials

Chitosan (mol wt. 340) and SA were purchased from Sigma-Aldrich, Castle Hill NSW, Australia, and OA from Fluka (Munich, Germany). To prepare chitosan emulsion (1.5%), chitosan powder (15 g) was dissolved in 1000 mL of 3% acetic acid solution and mixed well by using a magnetic stirrer and a hot plate at 50°C for 4

hours and allowed to cool at room temperature prior to its usage. The SA (2.0 mM) solution alone was prepared by dissolving 196 mg of SA powder in 600 mL of 3% acetic acid solution by using a magnetic stirrer at room temperature for 2 hours. To prepare 2.0 mM OA solution, 126 mg of OA powder was dissolved in 600 mL of 3% acetic acid solution by using a magnetic stirrer at room temperature for 2 hours. To prepare the chitosan emulsion (1.5%) loaded with SA (2.0 mM) or OA (2.0 mM), first SA or OA solution was prepared by using a magnetic stirrer hot plate at 50 °C for four hours followed by addition of Chitosan and Tween-20 (0.25%) as a surfactant. All the solutions/emulsions were adjusted to pH 5 by adding 0.1N sodium hydroxide and allowed to cool at room temperature. Fruit were then sprayed evenly with the specific edible coating material prepared fresh and allowed to dry at ambient conditions before transferring them to the specified storage conditions of the experiments.

3.3.3. Observations recorded

Data were recorded by following standard procedures described in detail in the respective chapters. The parameters were physiological characteristics- ethylene production, respiration rate; physical characteristics- weight loss, and firmness; biochemical properties- soluble solids concentration (SSC), titratable acidity (TA), ratio of SSC and TA; total and individual sugars and organic acids, ascorbic acid and total antioxidants.

3.3.4. Temperature and relative humidity recording

Temperature and relative humidity (RH) at the ambient conditions (Temp. 20 ± 2°C and R.H. 70 ± 5%), or cold storage (0 ± 1°C, 3 and 7°C, 95 ± 3% RH) were monitored by using Tinytag *Plus* Gemini Data Loggers (Gemini Data Loggers Ltd., Chichester, West Sussex, UK) interfaced to a computer with Tinytag Explorer software version 4.6.95 (Gemini Data Loggers Ltd., Chichester, West Sussex, UK). The data were recorded every 15 minutes.

3.4. Determination of ethylene production

Ethylene production was determined in nectarine, plum and citrus fruits by following the method of Pranamornkith et al. (2012) using a laser-based photoacoustic ethylene detector (ETD-300, Sensor sense B.V, Nijmegen, The Netherlands). The detector includes a set of valve controllers with an option of six valves connected to six

separate cuvettes [1.0 L air-tight jar, fitted with a rubber septum (SubaSeal, Sigma-Aldrich Co., St. Louis, USA)] (Fig. 3.2). Each fruit sample was weighed prior to shifting them into the cuvettes. To avoid any leakage, all the cuvettes were sealed very tightly. From each fruit sample, ethylene was estimated continuously for 20 min using an air flow rate of 4 L hr^{-1} and the average reading of ethylene production during the final 15 minutes was used for calculation. The "continuous flow" method was used with coarse mode (conversion factor 99818, capacity to measure ethylene concentration at 0-500 ppm, sensitivity at < 1%). The ethylene production rate (µl kg^{-1} h^{-1}) which was determined by Sensor sense was converted to µmol kg^{-1} h^{-1}.

Figure 3.2. Determination of ethylene production from the sample fruit by using ETD 300 ethylene detector.

Ethylene production was converted from µL kg^{-1} h^{-1} to µmol kg^{-1} h^{-1} using Ideal Gas Law, $PV = nRT$, where P is pressure (kPa), V is volume (L), n is the number of moles, R = 8.314 (the ideal gas constant) and T is temperature (Kelvin) (Bower et al.,

1998). Data of barometric pressure during the ethylene measurement were collected from Bureau of Meteorology Australia, WA. The relevant calculation is as follows-

We know in standard conditions-

The atmospheric pressure, P = 1 atm

Temperature, T = 273.15 K

Universal gas constant, R = 0.0821 L atm $mol^{-1}K^{-1}$

V = volume

N = Number of moles

In our case, we kept the fruits at 20°C, so, the temperature we need for calculation is

T = 273.15+20= 293.15K

Now, PV = nRT

=> V/n = RT/P

=> (0.0821 L atm $mol^{-1}K^{-1}$*293.15 K)/1 atm = 24.07 $Lmol^{-1}$

i.e., 1 mol gas = 24.07 L

or, 1 mmol gas = 24.07 ml

or, in 1 ml gas it has = 1/24.07 mmol = 0.0415 mmol

So, for example, if the measured ethylene gas is 2.5 ml $kg^{-1}hr^{-1}$, then there will be 2.5 X 0.0415= 0.104 mmol ethylene $Kg^{-1}hr^{-1}$.

3.5. Determination of rate of respiration

Respiration rate was determined on the basis of the amount of carbon dioxide (CO_2) produced from the treated and untreated fruits during ripening according to the method described earlier by Zaharah (2011). Headspace gas sample (2.0 mL) was taken through a rubber septum (SubaSeal®, Sigma-Aldrich Co., St. Louis, USA) fitted on 1L jar using a syringe and injected into an infrared gas analyzer [Servomex Gas Analyzer, Analyzer series 1450 Food Package Analyzer, Servomex (UK) Ltd., Crowborough, UK]. The respiration rate was calculated on the basis of the peak areas

of 2.0 mL gas sample and a CO_2 standard (Std CO_2, 8.52 ± 0.17%) (Fig. 3.3). The Std CO_2 was purchased from BOC Gases, Australia Ltd., (Perth, Australia). All the estimations were performed twice. Respiration rate was calculated by using the following formula and expressed as mL CO_2 kg^{-1} h^{-1}.

$$\text{Respiration rate (ml } CO_2 \text{ kg}^{-1} \text{ h}^{-1}) = \frac{\text{Changes in } CO_2 \text{ concentration (\%) X Vol. of container (L)}}{\text{Fruit weight (kg) X Incubation time (h)}}$$

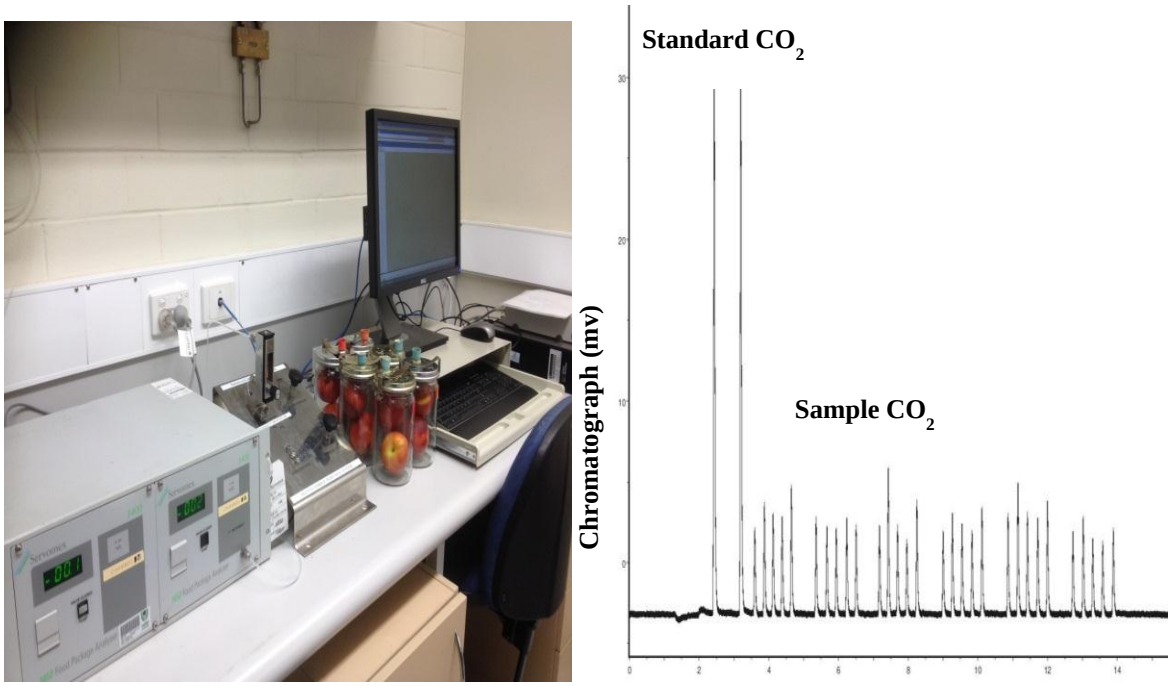


Figure 3.3. Determination of the rate of respiration and chromatograph of peak of standard (Standard CO_2), and fruit sample (Sample CO_2) by using an infrared gas analyzer (Servomex Gas Analyzer, Analyzer series 1450 Food Package Analyzer, Crowborough, England).

Respiration rate was converted from mL CO_2 kg^{-1} h^{-1} to mmol CO_2 kg^{-1} h^{-1} using Ideal Gas Law, PV = nRT as explained in Section 3.4. To check the possibility of CO_2 emission from the rubber septum or normal air, a blank injection from the headspace of the empty jar or air was also run under the similar conditions of analysis. No CO_2 emission was detected in blank injections.

3.6. Determination of loss of fruit weight

Fruit weight loss was expressed as percentage of fresh fruit weight against initial weight (g) at harvest (Ahmad et al., 2013) by using the following formula-

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

Fruit weight loss was estimated from each replication and expressed as a percentage.

3.7. Determination of fruit firmness

The firmness of fruit (nectarine and plum) and the compression strength of the citrus fruit were determined using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Fareham, UK) (Fig. 3.4) by following the methods detailed by Singh et al. (2009) and Hussain (2014). The texture profile analyser was equipped with a horizontal square base table (15 cm × 15 cm) and interfaced to a personal computer with Nexygen® software. A small slice (~2 mm thick) of fruit skin was removed and the firmness was recorded on opposite sides of the equatorial region of individual fruit by puncturing a 7/16 inch Magness-Taylor probe, using a 500 N load cell. The crosshead speed, depth, trigger and compression were maintained at 100 mm min^{-1}, 7.5 mm, 1 N and 75%, respectively, for all firmness determinations. In the case of compression test for sweet orange, fruit was placed between two flat plates with the stem axis perpendicular to the plate (Fig. 3.4). A crosshead speed of 200 mm min^{-1} and a strain of 50% of fruit height were maintained in the test. The peak force (newtons) that resulted from a sample being compressed to a given distance, time, or % of deformation (hardness 1) was recorded as fruit firmness (Fig.3.4). Fruit firmness was expressed in newtons (N).

3.8. Determination of soluble solids concentration (SSC), titratable acidity (TA) and SSC:TA ratio

Pulp (~15 g) from the inner and outer mesocarp at the middle of ten randomly selected fruits was used to extract juice using a fruit juicer (Model JE8500, Sunbeam Corp. Ltd., Botany, Australia). A digital refractometer (Atago-Palette PR 101, Atago Co., Itabashi-Ku, Tokyo, Japan) was used to determine the SSC from the extracted juice and was expressed as a percentage.

The TA of the extracted juice was determined as % malic acid (for nectarine and plum fruit) or % citric acid (for sweet orange fruit) by titrating the juice against 0.1 N NaOH using phenolphthalein as an indicator and was calculated by using the

following formula. SSC:TA ratio was calculated by dividing SSC with the corresponding TA value.

$$\text{Malic acid (\%)} = \frac{0.0067 \times \text{Vol. of NaOH} \times \text{Total vol (30)} \times 100}{\text{Volume of Juice (10)} \times \text{Volume of aliquot (5)}}$$

$$\text{Citric acid (\%)} = \frac{0.0064 \times \text{Vol. of NaOH} \times \text{Total vol (30)} \times 100}{\text{Volume of Juice (10)} \times \text{Volume of aliquot (5)}}$$

Where,

0.0067 = Milli-equivalent weight of malic acid

0.0064 = Milli- equivalent weight of citric acid

30 = Total volume (ml), 10 = Extracted juice sample (ml), 5 = Volume of aliquot (ml)

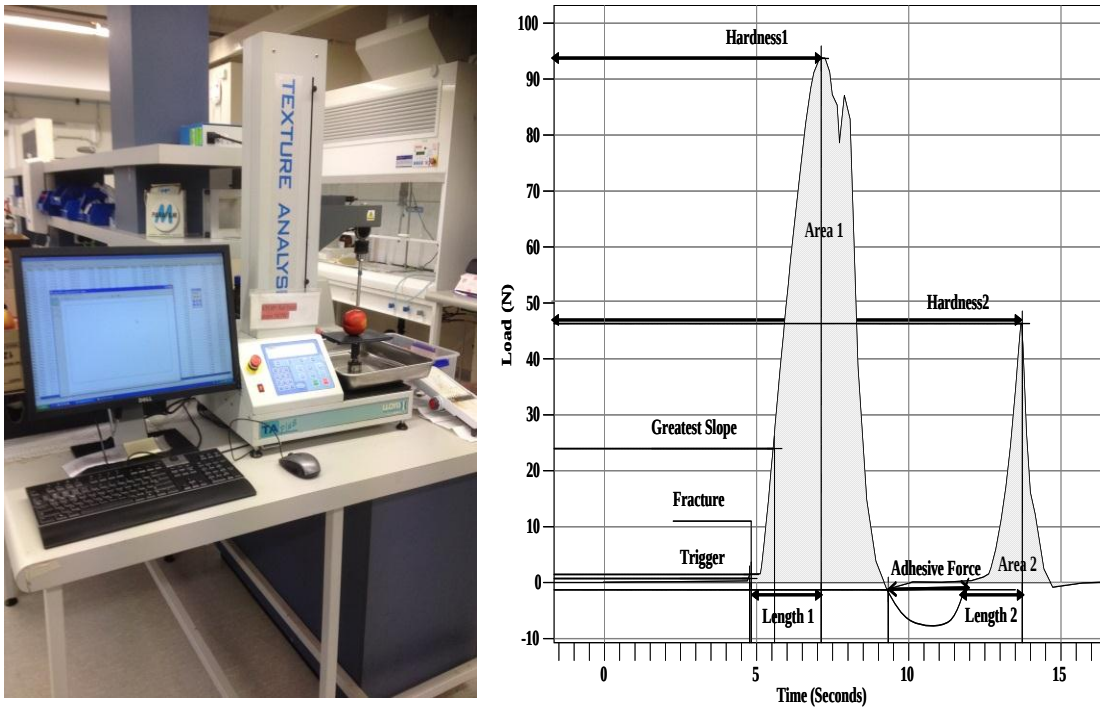


Figure 3.4. Determination of sample fruit firmness and graphical presentation of firmness profile of sample fruit using texture profile analyzer (TPA).

3.9. Determination of individual sugars and organic acids

3.9.1. Chemicals used

Individual standards used for determination of sugars (sucrose, D-glucose anhydrous and D-(-)-fructose) and organic acids (citric, malic, fumaric, succinic and tartrate acid) were of high-performance liquid chromatography (HPLC) grade and purchased from Sigma-Aldrich (Sydney, Australia).

3.9.2. Preparation of standards

Milli-Q water was used for preparing all the standards of individual sugars and organic acids. Sucrose (0.5g) and D-(-)-fructose (0.5g) was dissolved in 100 mL of water for preparing standard solutions of sucrose and fructose. Meanwhile, the standard solution for glucose was made by dissolving D-glucose (0.05 g) in 100 mL of water. The standard solutions of different organic acids were made by dissolving 0.1 g of citric, tartaric, succinic and 0.01 g of malic acid and fumaric acid in 100 mL of water. The standard solutions (4, 8, 12, 16 and 20 µL) were injected into the HPLC system maintaining similar settings and gradient as mentioned in the following Section 3.9.3.

3.9.3. Sample preparation

One ml fruit juice (FJ) was diluted with 19 ml of Milli-Q water, which was passed through a purification water system (Millipore, Bedford, MA, USA), to extract individual sugars and organic acids. The diluted juice was centrifuged at 12857× g for 15 minutes (Eppendorf Centrifuge 5810R, Hamburg, Germany) at 4ºC. Following the centrifugation, the diluted juice mixture (1 ml) was filtered through a 0.22 µm nylon syringe filter (Altech Associates, Baulkham Hills, Australia) and loaded into high-performance liquid chromatography system (HPLC) for determination of individual sugars and organic acids.

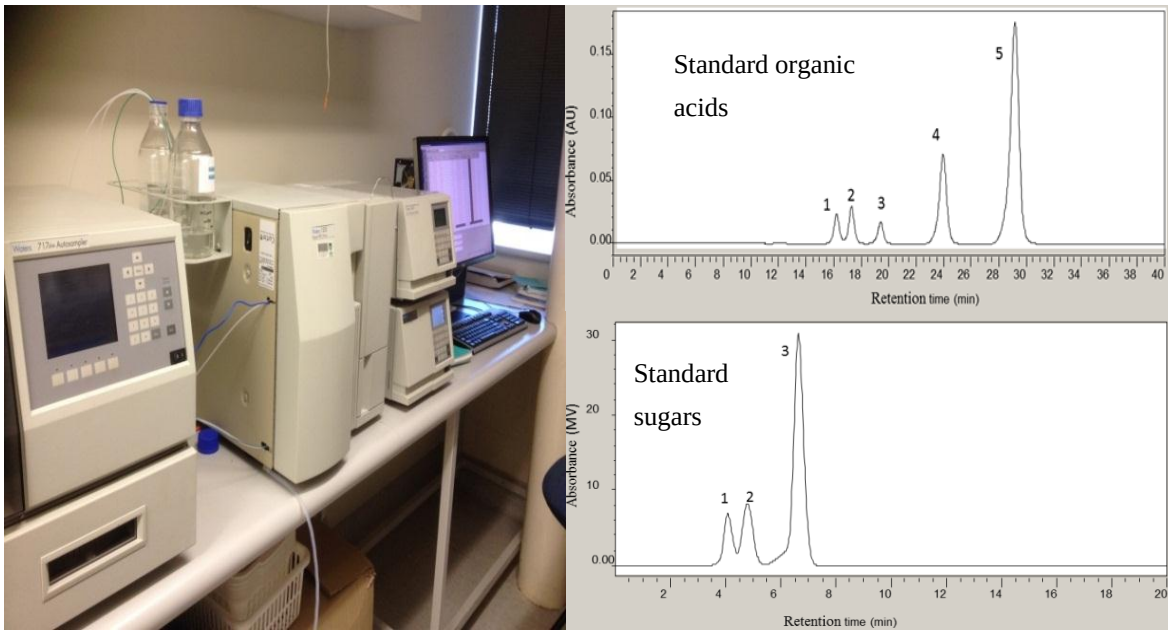


Figure 3.5. Determination of individual sugars and organic acids by using HPLC. Chromatographic profile of individual standard organic acids- (1) citric acid, (2) tartaric acid, (3) malic acid, (4) succinic acid and (5) fumaric acid (AU = Absorbance units); and sugars- (1) sucrose, (2) glucose and (3) fructose.

3.9.4. HPLC conditions

Individual sugars and organic acids in each sample were determined in duplicate using a reverse phase liquid chromatograph with a HPLC system (Waters 1525, Milford Corp., MA, USA) fitted to Dual λ Absorbance Detector (Waters 2487, Milford Corp., MA, USA) as previously detailed by Zaharah, (2011). An autosampler (Waters 717plus, Milford Corp., MA, USA) kept at 25°C, which was used to inject an aliquot (20 µl) of the sample (Fig. 3.3). The individual sugars and organic acids were separated on a Bio-Rad Aminex® HPX-87C Fast Carbohydrate column (100 × 7.8 mm) and Bio-Rad Aminex® HPX-87H column (300 × 7.8 mm) (Bio-Rad Laboratories, Inc., Hercules, USA) with a particle size of 9 µm, respectively. Both columns were headed by Cation H Bio-Rad Micro-Guard® column (30 × 4.6 mm) (Bio-Rad Laboratories, Inc., Hercules, USA). During the analysis, the temperatures of the Bio-Rad Aminex® HPX-87C Fast Carbohydrate column (for sugars), Bio-Rad Aminex® HPX-87H column (for organic acids) and guard column were maintained at 60°C and 45°C respectively during the analysis. The sulphuric acid solution (0.05 mM) was used as a mobile phase with the flow rate of 0.6 ml min^{-1} for elution of organic acids. Degassed water only (at the rate of 0.6 ml min^{-1}) was

used for eluting sugars. All individual organic acids were detected at 210 nm with dual wavelength UV detector. However, the individual sugars were detected using Refractive Index (RI) Detector (Water 2414, Milford Corp., MA, USA). The HPLC chromatographic peaks of different sugars and organic acids were identified by comparing the retention times with the standards. Breeze® 3.30 software (Waters, Milford Corp., MA, USA) was used to process the collected data (Fig. 3.5). All the individual sugars were calculated as g 100 g^{-1} FJ and different organic acids were expressed as g 100 g^{-1} FJ or mg 100 g^{-1} FJ depending upon their concentration.

3.10. Determination of vitamin C

The concentration of vitamin C in fruit samples was determined by following the method of Jagota and Dani (1982) and Malik and Singh (2005) with some modifications. Freshly extracted fruit juice (1 ml) from each replication was mixed with 5 ml of 6% metaphosphoric acid containing 0.18% ethylenediaminetetraacetic acid disodium salt (EDTA) and then homogenised and centrifuged at 1157 G for 15 minutes (Eppendorf Centrifuge 5810R, Hamburg, Germany) at room temperature. The supernatant (400 µl) was mixed with 200 µl of 3% metaphosphoric acid, 1.4 ml dH_2O and then 200 µl of diluted folin reagent (Folin: dH_2O, 1: 5 v/v) was added and the sample was mixed. After 15 min the absorbance was measured in duplicate at 760 nm wavelength using an UV/VIS Spectrophotometer (Model 6405, Felsted, Dunmow, UK). The concentration of vitamin C was calculated by using standard curve of L-ascorbic acid and expressed as mg ascorbic acid 100 ml^{-1} fruit juice.

3.11. Determination of total antioxidants

The levels of total antioxidants were determined by following the modified method of Brand-William et al. (1995) and Pham (2009) from the fruit juice (FJ). A stock solution containing 24 mg of DPPH (1, 1 diphenyl-2-picrylhydrazyl) prepared in 100 mL of 80% methanol and further diluted with methanol (1:4 v/v) to attain 1.1 absorbance at 515 nm to formulate a working solution (A). Aliquots of juice (50 µl) were mixed with 950 µl of the freshly prepared DPPH working solution (A), vortexed for 5 seconds and allowed to stand in the dark at 21 ± 1° C for 15 min. Then the absorbance of DPPH was measured at 515 nm by using an UV/VIS Spectrophotometer (Model 6405, Felsted, Dunmow, UK) and concentrations of total antioxidants were calculated using a standard curve of 6-hydroxy-2, 5, 7, 8-

tetramethylchromane-2-carboxylic acid (Trolox) and was expressed as µM trolox equivalent antioxidant activity (TEAC) 100 ml^{-1} FJ basis.

3.12. Estimation of chilling injury index (CI)

The chilling injury index was determined by ranking the individual fruit using a rating scale from 0 to 3; (0 = normal, 1 = slight, 1 - 25% on fruit surface, 2 = moderate, 25 - 50% on fruit surface and 3 = severe, > 50% on fruit surface). The method was described earlier by Cohen et al. (1994). The following formula was used to determine chilling injury index.

$$\text{Chilling injury index (CI)} = \frac{\sum (\text{Injury level X number of fruit at each level})}{\text{Total number of fruit}}$$

3.13. Determination of disease incidence

The disease incidence was determined by examining the fruit regularly following each storage period by following method described earlier by El-Ghaouth et al. (1991). The fruits showing visible symptoms of disease counted as diseases. Disease incidence was expressed as percentage of fruit infected and calculated as follows.

$$\text{Disease incidence (\%)} = \frac{(\text{Total number of fruits} - \text{Infected fruits}) \text{ x } 100}{\text{Total number of fruits}}$$

3.14. Statistical Analysis

The data from various experiments were analysed by one or two-way analysis of variance (ANOVA) using Genstat 14th edition (release 14.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK). Fisher's least significant differences (LSD) was calculated following a significant ($P \leq 0.05$) F test. LSD was used to check the significant differences between the treatments. The validity of statistical analysis was tested by checking all the assumptions of ANOVA.

CHAPTER 4

Effects of chitosan emulsion, salicylic acid or oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid on postharvest quality of nectarine (*Prunus persica* L. Batch. cv nectarine) fruit at ambient temperature

Summary

Edible coatings are used to improve the attractive appearance, extend shelf life and maintain fruit quality by acting as a barrier against moisture and gaseous exchange during postharvest handling and storage. Chitosan, salicylic acid (SA) and oxalic acid (OA) are the widely used edible coatings. However, the effect of chitosan emulsion, (SA), (OA) alone and chitosan emulsion loaded with SA or OA on ethylene production and fruit quality of nectarine has not yet been investigated. The current study was conducted to elucidate the effect of chitosan emulsion, SA or OA alone and their combinations on modulating fruit ripening and quality of white flesh nectarine cultivars 'Honey Fire' and 'Bright Pearl' under ambient conditions. 'Honey Fire' and 'Bright Pearl' nectarine fruits showed genotypic differences in response to the edible coatings used in the experiment. Fruit coated with chitosan (1.5%) emulsion showed suppressed mean ethylene production (0.07 µmol Kg^{-1} h^{-1}), higher level of fumeric acid (17.35 mg 100 ml^{-1} FJ), sucrose (9.74 g 100 ml^{-1} FJ) and total sugars (11.84 g 100 ml^{-1} FJ) in ripe 'Honey Fire' nectarine compared to the control and all other treatments. Fruit coated with the chitosan emulsion loaded with 2.0 mM SA maintained higher level of vitamin C (14.75 mg 100 ml^{-1} FJ), firmness (14.19 N), soluble solids concentration (SSC) (17.57%), SSC: Titratable acidity (TA) ratio (13.16) and tartaric acid (23.00 mg 100 ml^{-1} FJ) in ripe 'Honey Fire' nectarine fruit. Highest levels of antioxidants were recorded in both cultivars of nectarine fruit treated with 2.0 mM SA alone (46.78 and 48.13 µM Trolox equiv., 100 ml^{-1} FJ). Higher level of SSC:TA ratio (18.87), reduced loss of weight (5.46%), higher level of fumeric acid (7.65 mg 100 ml^{-1} FJ), malic acid (535.6 mg 100 ml^{-1} FJ), succinic acid (4.09 mg 100 ml^{-1} FJ), tartaric acid (51.67 mg 100 ml^{-1} FJ) and total organic acid (1.20 g 100 ml^{-1} FJ); and higher level of sucrose (11.14 g 100 ml^{-1} FJ), fructose (1.73 g 100 ml^{-1} FJ) and total sugars (14.33 g 100 ml^{-1} FJ) were noted in chitosan coated 'Bright Pearl' compared to control and all other treatments. Highest level of

SSC (15.47%) and TA (0.98%) was recorded in OA treated 'Bright Pearl' nectarine fruit. Suppressed ethylene production (0.75 µmol kg^{-1} h^{-1}) and highest firmness (23.85 N) was noted from combined treatment of chitosan and SA in 'Bright Pearl' nectarine fruit compared to the control and other treatments. In conclusion, the response of nectarine fruit to different coating treatments in maintaining various fruit quality in ambient temperature was genotype dependent. Coating treatments of chitosan emulsion (1.5%) loaded with SA (2.0 mM) or OA (2.0 mM) were more effective in maintaining many fruit quality parameters in 'Honey Fire' nectarine fruit compared to chitosan emulsion, SA and OA alone at ambient temperature and the trend was reversed in cultivar 'Bright Pearl'.

4.1. Introduction

The nutritional value and the protective role of fruits against various diseases like inflammation, cardiovascular, cancer and aging related disorders has attracted the attention of consumers (Huang et al., 2008). Nectarine is a rich source of different kinds of vitamins, minerals and antioxidative compounds (Gil et al., 2002). Nectarine is a climacteric fruit with a very limited storage life (2 to 5 weeks). Nectarine fruit exhibits increased ethylene production and rate of respiration, significant changes in fruit texture, colour, aroma, and other biochemical and physiological attributes during ripening (Lill et al., 1989). Various techniques have been reported to delay post-storage ripening of nectarine fruit with limited success. These techniques include pre- and postharvest application of calcium (Manganaris et al., 2006) and postharvest heat treatment (Obenland et al., 2005); use of 1- methylcyclopropene (1-MCP) (Liguori et al., 2004), AVG (aminoethoxyvinylglycine) (Garner et al., 2001) or *Aloe vera* gel coating (Ahmed et al., 2009). Beneficial effects of controlled, modified atmosphere (Akbudak and Eris, 2004; Uthairatanakij et al., 2005) and cold storage (Manganaris et al., 2005a) on extending storage life and maintaining quality of nectarine fruit have also been reported. Nectarine fruit show physiological disorders such as chilling injury if not stored at safe cold storage temperature (0 - 1°C) (Manganaris et al., 2005a).

Various edible coating materials (alginate, cellulose, chitosan, chitin, lipids, mucilage, milk protein, starch, wax, and zein) act as a barrier to loss of moisture and diffusion of oxygen during postharvest handling and storage of fruit. Edible coatings

are used to improve the attractive appearance of the commodity, extend shelf life and maintain fruit quality but exhibited varying success (Baldwin et al., 1995; Petersen et al., 1999; Cha and Chinnan, 2004; Valverde et al., 2005). The edible coating materials are well accepted due to their environmentally friendly nature, natural biocide activity, and ability to create an atmosphere similar to modified atmosphere packaging (MAP) (Cha and Chinnan, 2004). Though waxes are widely used as coating material they are equally effective for a range of fruits (Cha and Chinnan, 2004). Lipid / hydrocolloid coatings have been reported to maintain consistent fruit firmness, crispness and juiciness following 8 weeks cold storage of 'Golden Delicious' apple (Conforti and Totty, 2007). Romanazzi et al. (2003) reported that chitosan coatings reduce postharvest decay in various fruit crops. Moreover, chitosan coating has been used to prolong shelf life and inhibit postharvest decay of many fruits such as peach, citrus, strawberry, table grape and litchi (Zhao et al., 2006). Giacalone and Chiabrando (2015) reported that 'Diamond Ray' nectarine fruit that have been coated by chitosan solution and stored at 0°C for 30 days showed high level of soluble solids, titratable acidity and texture values.

Salicylic acid (SA) is a safe and natural endogenous phenolic compound in plants and is known to reduce ethylene production, respiration rate, prevent oxidative stresses, retard fruit ripening and senescence, induce disease resistance and reduce postharvest losses of horticultural commodities (Asghari and Aghdam, 2010). SA is one of the main phenolic compounds that have been reported to instigate a number of physiological processes in plants including ethylene production, regulation of plant growth, development of sex polarization, seed germination and disease resistance (Babalar et al., 2007; Asghari and Aghdam, 2010; Al-Qurashi and Awad, 2012). Khademi and Ershadi (2013) reported that postharvest dip treatment of SA (2.0 mM) for five minutes improved peach fruit firmness and lowered weight loss and fruit decay when stored in cold conditions (0 ±1 °C) for 42 days. Furthermore, SA (0.8 mM) has been found to decrease respiration rate, ethylene production and increase the activity of antioxidant enzymes in sugar apple fruit (*Amona squamosa* L.) (Mo et al., 2008).

Zheng et al (2007b) reported that postharvest application of OA (5.0 mM) for 18 days reduced ethylene production, delayed the loss of firmness and ripening in mango fruit (*Mangifera indica* L. cv. Zill) at ambient conditions. Postharvest dip

application of OA (1.0 and 5.0 mM) for 10 minutes reduced respiration rate and increased activity of antioxidant enzymes, reduced softening and delayed ripening in 'Bayuecui' peach fruit (Zheng et al., 2007a). Some effects of chitosan, SA and OA alone on peach, citrus, strawberry, mango, sugar apple, and litchi fruits have been reported. However the effects of postharvest application of chitosan, loaded with SA or OA on the modulation of ethylene production, respiration and changes in SSC, TA, SSC:TA ratio, vitamin C and total antioxidants during ripening fruit quality of nectarine fruit have not yet been investigated. Therefore in this study we evaluated whether chitosan loaded with SA or OA is more effective compared to their individual application in suppressing ethylene production, fruit ripening and quality of white flesh nectarine cultivars 'Honey Fire' and 'Bright Pearl' under ambient conditions.

4.2. Materials and methods

4.2.1. Plant material

Nectarine (*Prunus persica* L. Batsch cv. Honey Fire and Bright Pearl) fruit were harvested at commercial maturity (SSC = 12.45% and 12.48%, fruit firmness = 64.29 N and 71.28 N, ethylene production = 0.048 and 0.054 µmol kg^{-1} h^{-1} respectively) from Casuarina Valley Orchard, Karagullen, Perth Hills (31° 57′S; 115° 50′E), Western Australia. Fruit of uniform size, free from visible symptoms of disease were transported to the Horticulture Research Laboratory, Curtin University, Perth, WA, within one hour of harvest.

4.2.2. Treatments and experimental design

In the first experiment, the fruit of nectarine cv. Honey Fire were coated by spraying an aqueous emulsion of chitosan emulsion (1.5%), or a solution of SA (2.0 mM) or OA (2.0 mM) alone or the chitosan emulsion (1.5%) loaded with SA (2.0 mM) or OA (2.0 mM) and Tween 20 (0.25%) as a surfactant in each solution. Uncoated fruit served as a control. Following the treatments, the fruit were kept at ambient conditions (20 ± 1° C and 60 ± 5% RH). Ethylene production from the fruit was determined on day 0, 2, 4 and 6 after the treatments. Fruit weight loss was recorded 7 days after treatment. Meanwhile, firmness, soluble solids concentration (SSC), titratable acidity (TA), ratio of SSC and TA, total and individual sugars and organic acids, vitamin C and total antioxidants were determined three and seven days after

treatments. The experiment followed completely randomized design (CRD) with four replications and 10 fruits in each replication. In the second experiment, mature fruit of 'Bright Pearl' cultivar of nectarine were treated and evaluated in the same manner as in the first experiment 1, but ethylene production from the fruit was determined daily for seven days following treatments. All other parameters were determined seven days after treatments.

4.2.3. Determination of production of ethylene

Ethylene production was determined by following the method described earlier by Pranamornkith et al. (2012) and detailed in Chapter 3, Section 3.4. The level of ethylene was determined by using an ETD 300 ethylene detector (Sensor sense B.V, Nijmegen, The Netherlands). The production of ethylene was expressed as μmol kg^{-1} h^{-1}.

4.2.4. Determination of loss of fruit weight

Fruit weight loss was calculated as the percentage of fresh fruit weight against initial weight at harvest as reported by Ahmad et al. (2013) and also described in detail in Chapter 3, Section 3.6.

4.2.5. Determination of fruit firmness

The firmness of fruit pulp was determined using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK) equipped with a horizontal square base table (15 cm × 15 cm) and by following the methods explained earlier by Singh et al. (2009) and detailed in Chapter 3, Section 3.7. Fruit firmness was expressed as newtons (N).

4.2.6. Determination of SSC, TA and SSC:TA ratio

The SSC, TA and their ratio were determined from the nectarine fruit juice extracted from the pulp (~15 g) of 10 randomly selected fruit and by using a fruit juicer (Model JE8500, Sunbeam Corp. Ltd., Botany, Australia). A digital refractometer (Atago-Palette PR 101, Atago Co., Itabashi-Ku, Tokyo, Japan) was used to determine the SSC from the extracted juice and was expressed as a percentage. TA was determined by titrating the juice against 0.1 N NaOH using phenolphthalein as an indicator. TA was expressed as % malic acid. SSC:TA ratio was calculated by dividing SSC by the corresponding TA value. Details of the procedures have been described in Chapter 3, Section 3.8.

4.2.7. Determination of individual sugars and organic acids

Individual sugars were determined by using HPLC system (Waters 1525, Milford Corp., MA, USA) with Bio-Rad Aminex® HPX-87C Fast Carbohydrate column (100 × 7.8 mm) and a Refractive Index (RI) Detector (Water 2414, Milford Corp., MA, USA). Individual organic acids were separated using HPLC system (Waters 1525, Milford Corp., MA, USA) with Bio-Rad Aminex® HPX-87H column (300 × 7.8 mm) (Bio-Rad Laboratories, Inc., Hercules, USA) and a Dual λ Absorbance Detector (Waters 2487, Milford Corp., MA, USA). The data were collected and processed with Breeze® 3.30 software (Waters, Milford Corp., MA, USA). The concentrations of individual sugars such as sucrose, fructose and glucose were expressed as g 100^{-1} FJ and organic acids as g 100^{-1} FJ or mg 100^{-1} FJ. The detailed method has also been described in Chapter 3, Section 3.9.

4.2.8. Determination of vitamin C

Vitamin C concentrations were estimated using the method previously described by Malik and Singh (2005) using a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, Dunmow, Essex, UK). Vitamin C concentration was expressed as mg vitamin C 100 ml^{-1} FJ. The detailed method has also been explained in Chapter 3, Section 3.10.

4.2.9. Determination of total antioxidants

Total antioxidants were determined by employing the method described by Pham (2009), which was modified from methodof Brand-William et al. (1995). Total antioxidants were expressed as µM trolox equivalent antioxidant activity (TEAC) 100 ml^{-1} FJ. The detailed method has also been described in Chapter 3, Section 3.11.

4.2.10. Disease incidence

The disease incidence was expressed as a percentage and determined by examining the fruit regularly and regarded as infected if a visible lesion was observed. The detailed method has also been included in Chapter 3, Section 3.13.

4.2.11. Statistical analysis

The experimental data were subjected to one-way or two-way analysis of variance (ANOVA), using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted

experimental station, UK). The effects of different coating treatments, fruit ripening period and their interactions on different parameters were assessed within ANOVA and the least significant differences were calculated following significance F test at $P \leq 0.05$.

4.3. Results

4.3.1. Ethylene production

When averaged over ripening time, chitosan emulsion (1.5%) coating significantly ($P \leq 0.05$) suppressed mean ethylene production (0.39-fold) compared to the control (0.18 µmol kg^{-1} h^{-1}) and other treatments in 'Honey Fire' nectarine fruit (Fig. 4.1A). Meanwhile, the treatment of chitosan (1.5%) emulsion loaded with SA (2.0 mM) suppressed mean ethylene production (0.65-fold) during ripening period in 'Bright Pearl' nectarine fruit in comparison to the control fruit (1.15 µmol kg^{-1} h^{-1}) and other treatments (Fig. 4.1B). The treatment of chitosan emulsion (1.5%) was most effective in reducing climacteric ethylene production in 'Honey Fire' nectarine fruit during the ripening period followed by SA (2.0 mM) and OA (2.0 mM) alone compared to all other treatments and control (Fig. 4.2A). In cultivar 'Bright Pearl', the fruit coated with emulsion of chitosan (1.5%) loaded with SA (2.0 mM) showed suppressed climacteric ethylene production compared to control and all other treatments (Fig. 4.2B).

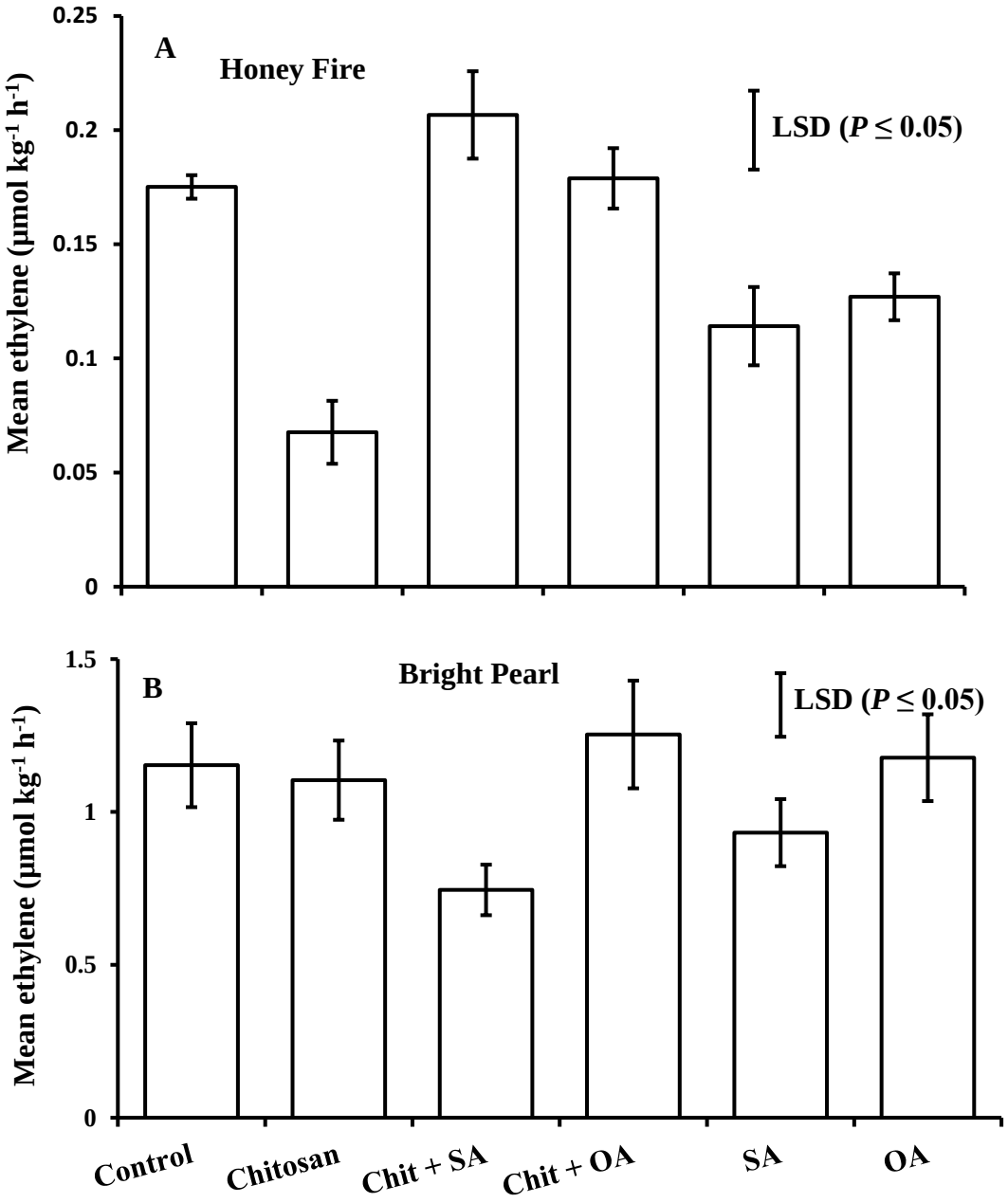


Figure 4.1. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on mean ethylene production when averaged over fruit ripening period in (A) 'Honey Fire' and (B) 'Bright Pearl' cultivars of nectarine. Vertical bars represent SE, n = four replicates, two fruit in each replication.

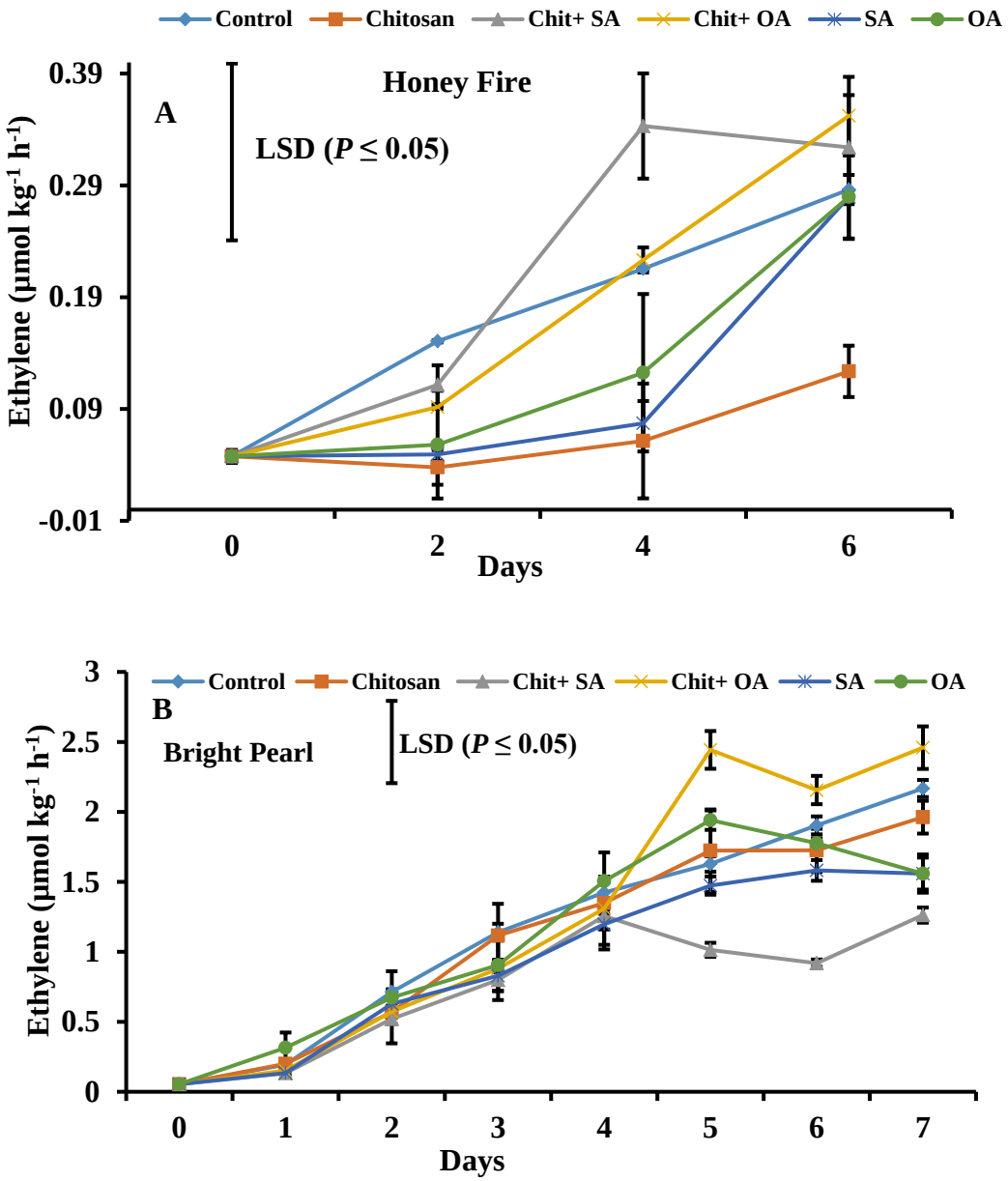
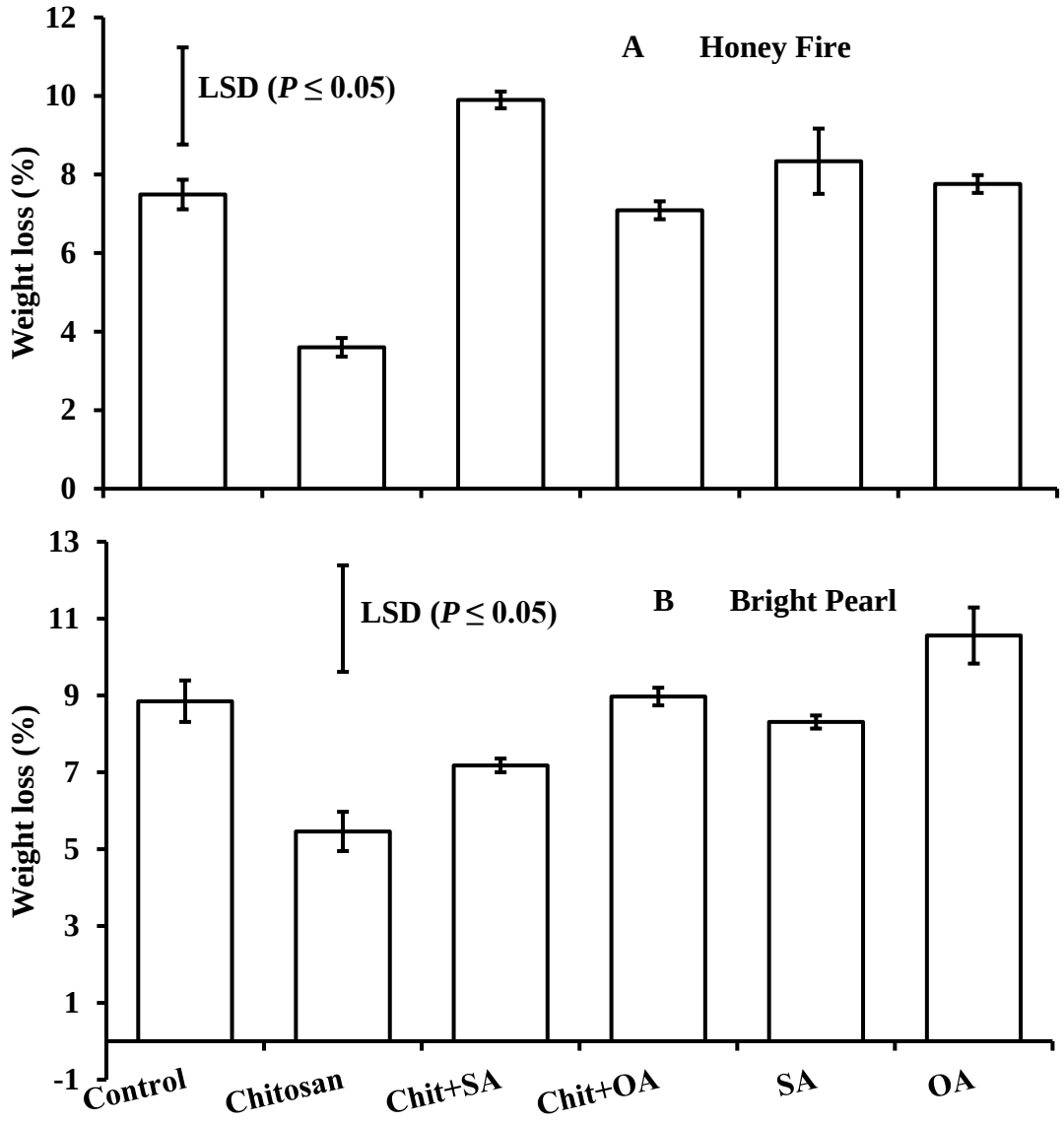


Figure 4.2. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on ethylene production during fruit ripening period in (A) 'Honey Fire' and (B) 'Bright Pearl' cultivars of nectarine. Vertical bars represent SE, n = four replicates, two fruit in each replication.

4.3.2. Weight loss

Chitosan coated fruit in both 'Honey Fire' and 'Bright Pearl' nectarine cultivars exhibited significantly ($P \leq 0.05$) least weight loss (3.6% and 5.46% respectively) than the control (7.49% and 8.85% respectively) and other treatments (Fig. 4.3A and B). The loss of weight was highest when fruit were coated with chitosan emulsion loaded with SA (9.9%) followed by SA alone (8.34%) and OA alone (7.76%) in 'Honey Fire' nectarine fruit (Fig. 4.3A). However, the highest loss of weight in

'Bright Pearl' nectarine fruit was recorded in the fruit coated with OA alone (10.56%) followed by the treatment of chitosan emulsion loaded with OA (8.97%) and control fruit (8.85%) (Fig. 4.3B).



Figre 4.3. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on weight loss during fruit ripening period in (A) Honey Fire and (B) Bright Pearl cultivars of nectarine. Vertical bars represent SE, n = four replicates, ten fruit in each replication.

4.3.3. Firmness

'Honey Fire' nectarine fruit coated with chitosan emulsion loaded with SA exhibited significantly ($P \leq 0.05$) higher fruit firmness (24.35 and 14.19 N) on the third and seventh day after treatment compared with the control and all other treatments

respectively (Fig. 4.4A). The treatment of chitosan emulsion loaded with SA and chitosan emulsion alone resulted in significantly highest firmness (23.85 and 18.78 N respectively) in ripe 'Bright Pearl' nectarine fruit as compared to the control and other treatments. Firmness was lowest in control fruit (3.65 N) at ripe stage in 'Bright Pearl' nectarine compared to all other treatments (Fig 4.4B).

4.3.4. Soluble solids concentration (SSC)

'Honey Fire' nectarine fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) exhibited significantly higher SSC (16.42% and 17.57%) at three and seven days after treatment respectively compared with control and all other treatments (Fig. 4.5A). Fruit coated with OA (2.0 mM) showed lowest SSC (12.67% and 12.65%) at three and seven days after treatment respectively compared with control and all other treatments. Meanwhile, 'Bright Pearl' nectarine fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) showed the lowest SSC (13.01%) after seven days of ripening (Fig. 4.5B). The highest SSC was recorded in ripe 'Bright Pearl' nectarine fruit which were coated with 2.0 mM OA alone (15.47%).

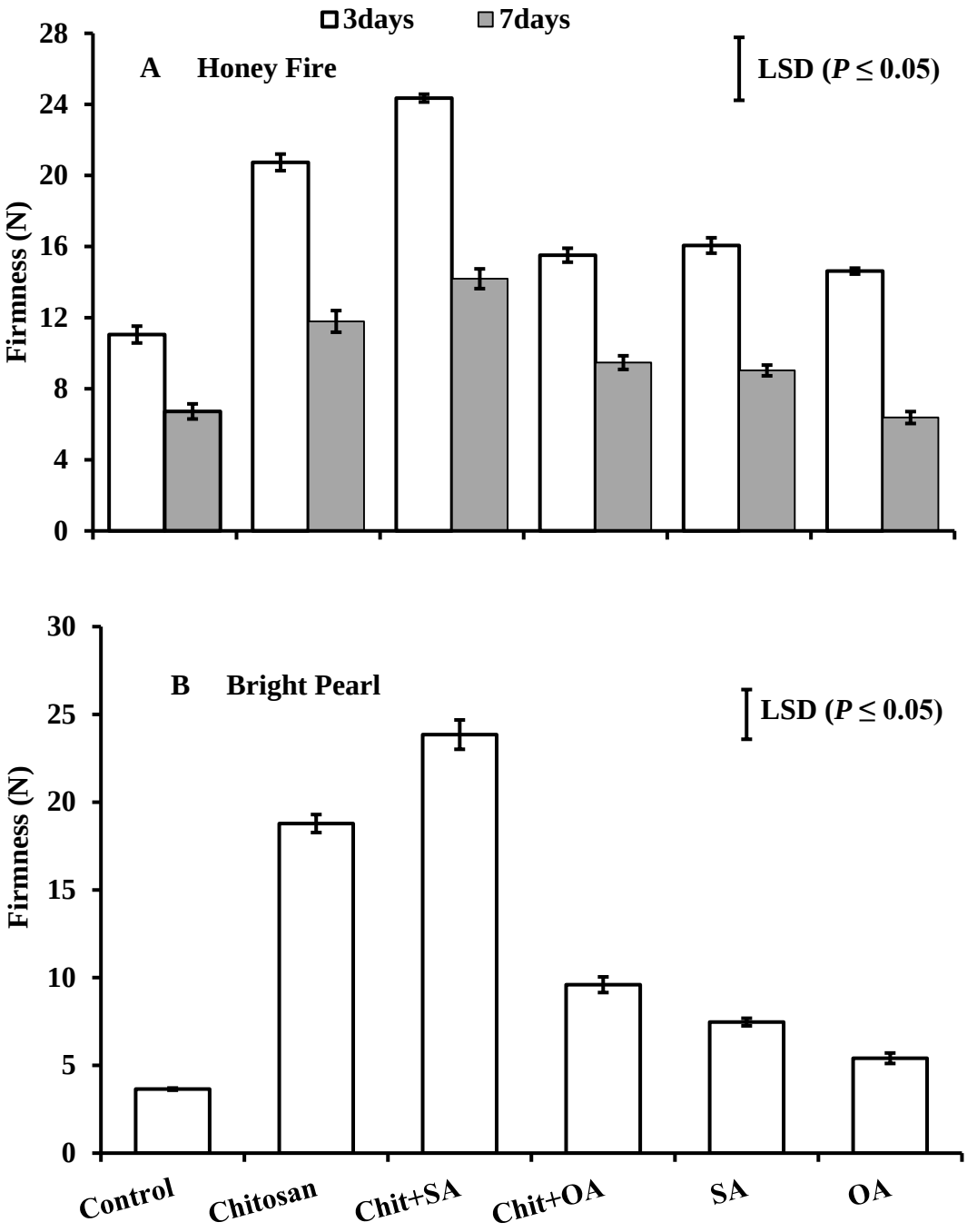


Figure 4.4. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on fruit firmness during fruit ripening period in (A) Honey Fire and at ripe stage in (B) Bright Pearl cultivar of nectarine. Vertical bars represent SE, n = four replicates, ten fruit in each replication.

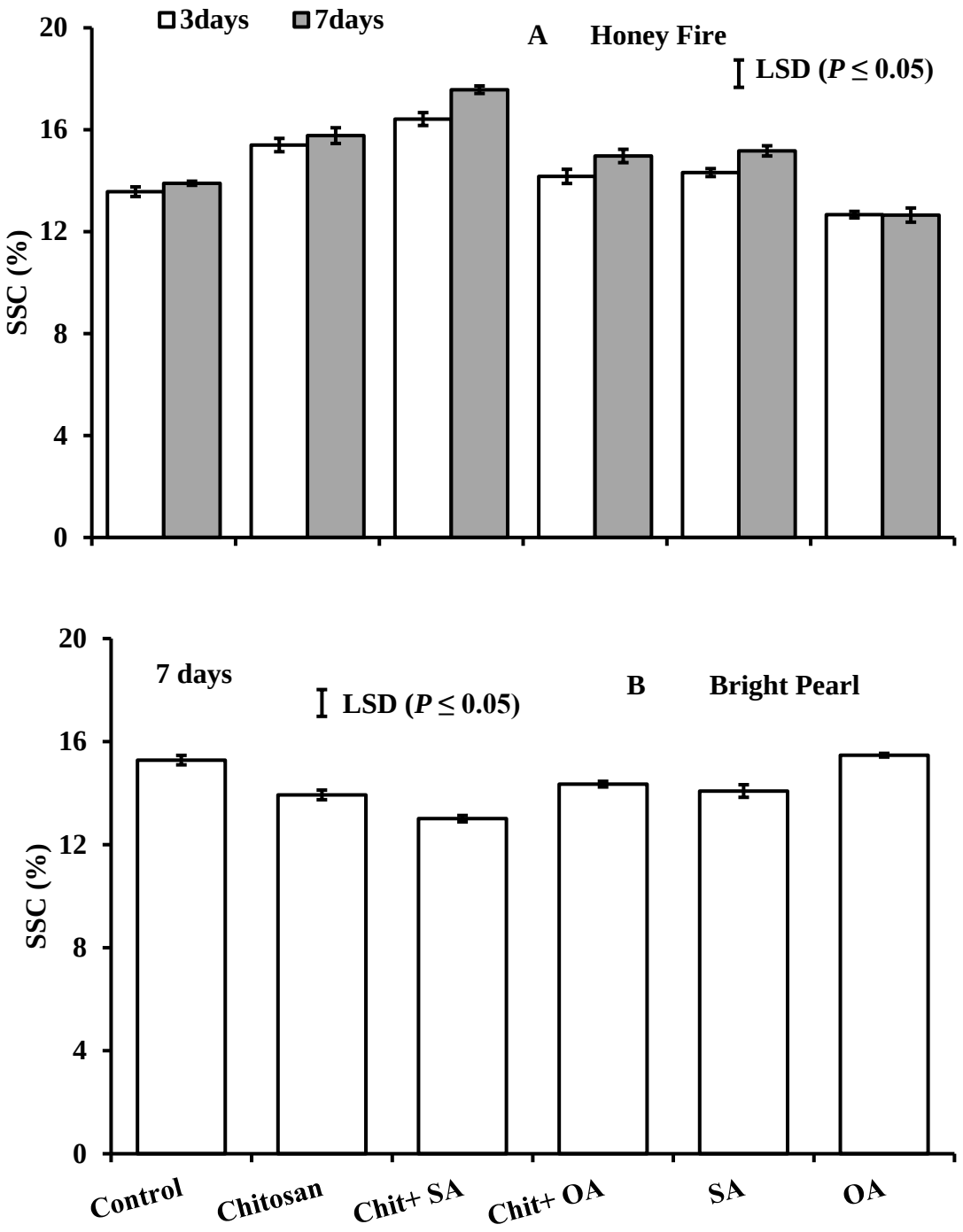


Figure 4.5. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on soluble solids concentration (SSC) during fruit ripening period in (A) Honey Fire and at ripe stage in (B) Bright Pearl cultivar of nectarine. Vertical bars represent SE, n = four replicates, ten fruit in each replication.

4.3.5. Titratable acidity (TA)

'Honey Fire' nectarine fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) exhibited a significantly higher level of TA (1.52%) than the control

(1.12%) and all other treatments after three days of treatment (Fig. 4.6A). On the seventh day after treatment, highest levels of TA (1.54 and 1.53%) were recorded in 'Honey Fire' nectarine fruit which were coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) or OA alone respectively (Fig 4.6A). Similarly, higher level of TA was recorded after seven days of treatment in the 'Bright Pearl' nectarine fruit coated with OA (2.0 mM) alone (0.98%) followed by control (0.92%), chitosan emulsion loaded with SA (0.89%) or OA (0.86%) (Fig. 4.6 B).

4.3.6. SSC:TA ratio

The SSC:TA ratio in 'Honey Fire' nectarine fruit coated with chitosan emulsion (1.5%) showed highest SSC:TA ratio (12.72) on the third day after treatment compared to control and all other treatments, whilst OA coated fruit showed lower SSC:TA ratio (9.54) (Fig. 4.7A). 'Honey Fire' nectarine fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) resulted in significantly highest SSC: acid ratio (13.16) on the seventh day after treatment compared to control and all other treatments. However, the lowest SSC:TA ratio (14.75) was recorded in 'Bright Pearl' nectarine fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) followed by OA alone (15.89), control (16.64) and chitosan emulsion loaded with OA (16.69). The highest SSC:TA ratio (18.87) was recorded in 'Bright Pearl' nectarine fruit coated with 1.5% chitosan emulsion alone as compared to the control and all other treatments (Fig. 4.7B).

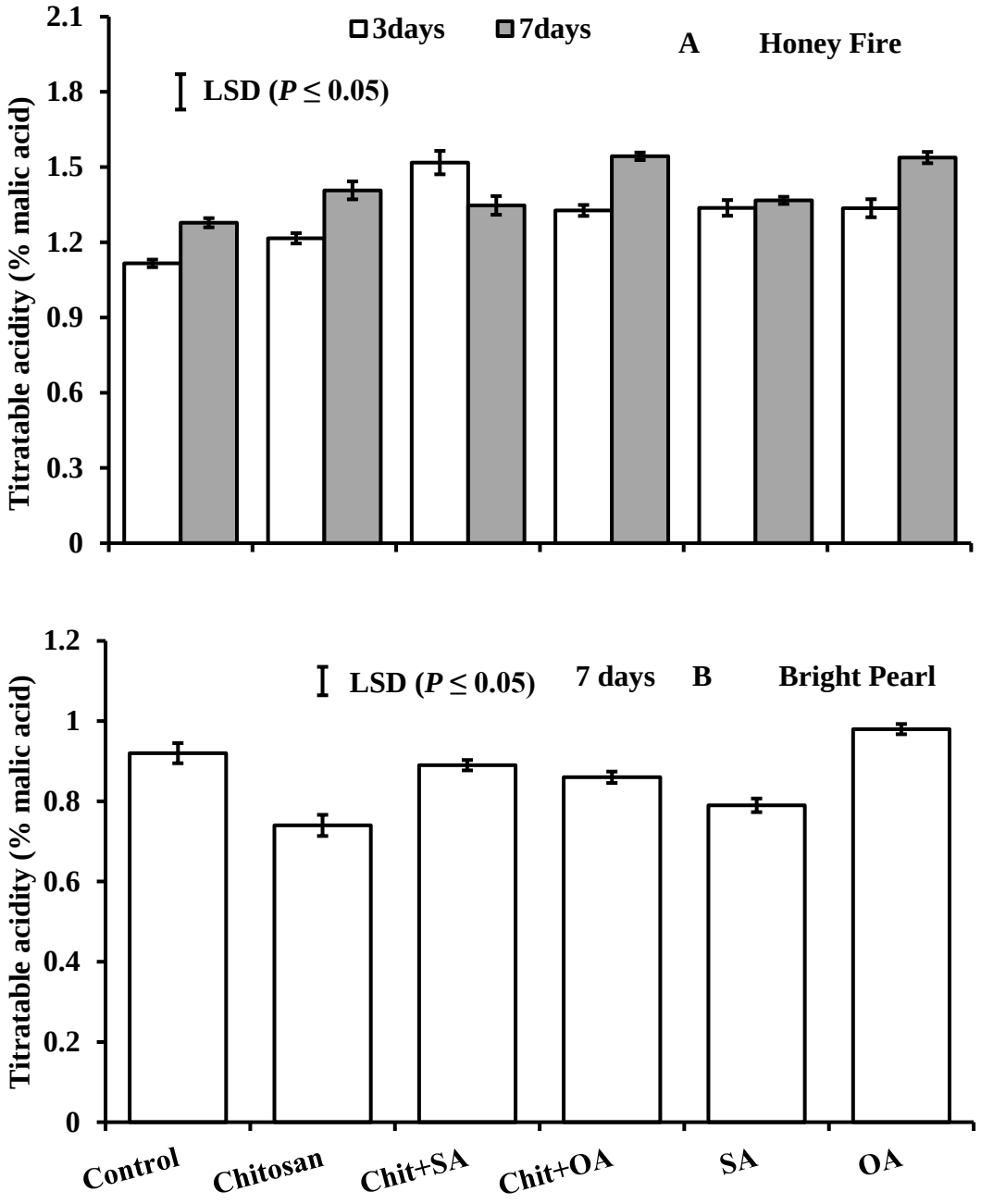


Figure 4.6. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on titratable acidity (TA) during fruit ripening period in (A) Honey Fire and at ripe stage in (B) Bright Pearl cultivar of nectarine. Vertical bars represent SE, n = four replicates, ten fruit in each replication.

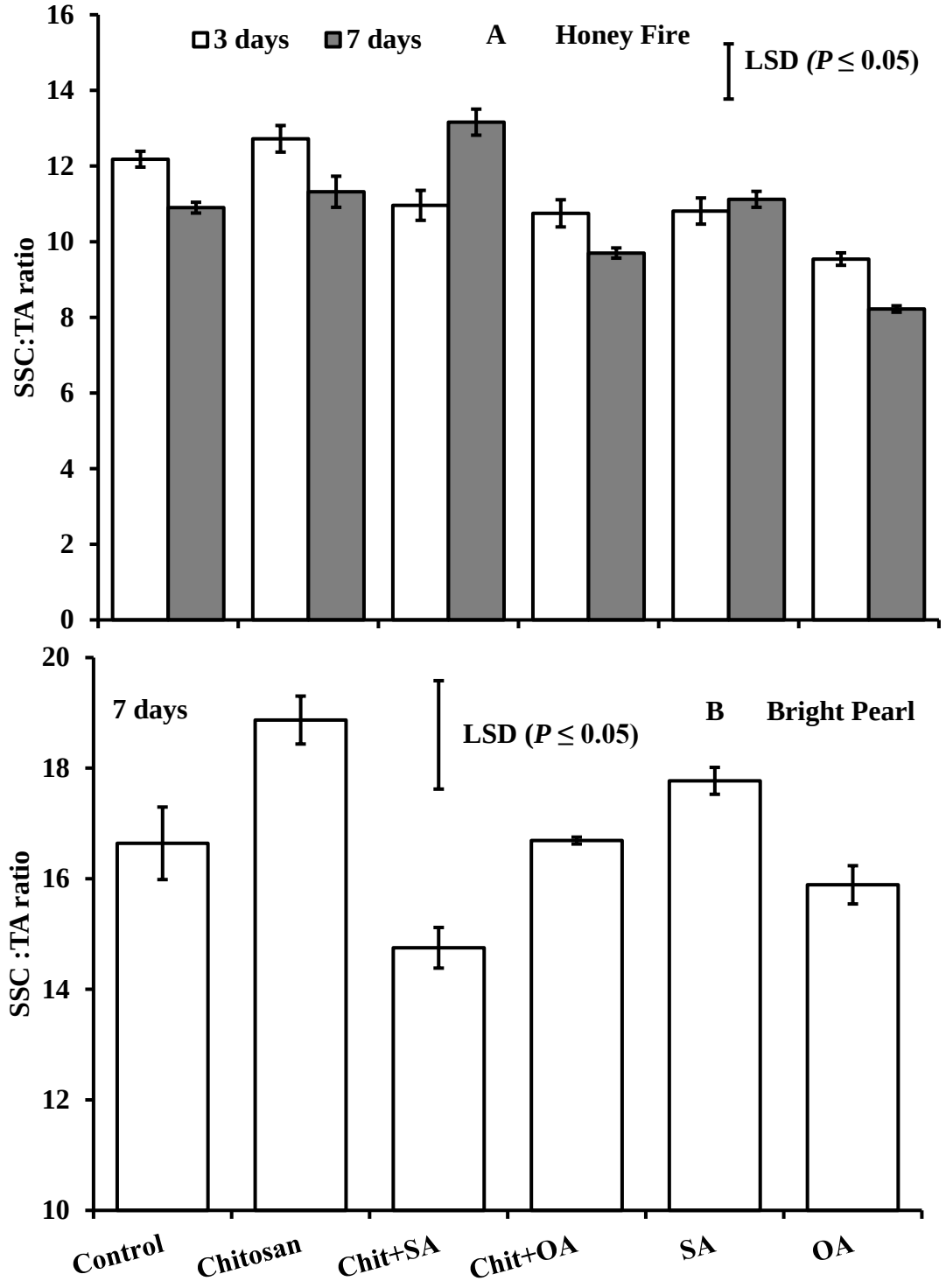


Figure 4.7. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on SSC:TA ratio during fruit ripening period in (A) Honey Fire and at ripe stage in (B) Bright Pearl cultivar of nectarine. Vertical bars represent SE.

4.3.7. Sugars:

Sucrose was found to be the predominant sugar in both 'Honey Fire' and 'Bright Pearl' nectarine fruit, followed by fructose and glucose.

4.3.7.1. Fructose

When averaged over ripening time, 'Honey Fire' nectarine fruit coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) resulted in significantly ($P \leq 0.05$) higher mean concentrations of fructose (2.02 g $100g^{-1}$ FJ) compared to control and all other treatments (Table 4.1). When averaged over treatments, mean concentration of fructose significantly ($P \leq 0.05$) increased from day three (1.63 g $100g^{-1}$ FJ) to seven days after treatment (1.75 g $100g^{-1}$ FJ) in 'Honey Fire' nectarine fruit. The interaction between the treatments and the ripening period was found to be non-significant for levels of fructose. The levels of fructose in 'Bright Pearl' ripe nectarine were significantly ($P \leq 0.05$) higher (1.73 and 1.55 g $100g^{-1}$ FJ) when coated with chitosan emulsion (1.5%) followed by chitosan emulsion (1.5%) loaded with SA (2.0 mM) respectively as compared to the control and all other treatments (Fig 4.8A). 'Bright Pearl' ripe nectarine fruit coated with OA (2.0 mM) alone exhibited the lowest level of fructose (1.01 g $100g^{-1}$ FJ) as compared to all other treatments (Fig. 4.8A).

4.3.7.2. Glucose

'Honey Fire' nectarine fruit coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) resulted in significantly ($P \leq 0.05$) higher mean concentrations averaged over ripening time of glucose (0.62 g 100g-1 FJ) compared to control and all other treatments. When averaged over treatments, mean concentration of glucose increased significantly ($P \leq 0.05$) from three to seven days after treatment (0.47 and 0.57 g $100g^{-1}$ FJ) respectively in 'Honey Fire' nectarine fruit. The interaction between different treatments and the ripening period was found to be significant for levels of glucose (Table 4.1). The level of glucose was significantly ($P \leq 0.05$) higher (1.85 g $100g^{-1}$ FJ) in ripe 'Bright Pearl' nectarine fruit, which were coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) as compared to the control and all other treatments (Fig. 4.8B). The ripe fruit which were coated with OA (2.0 mM) exhibited the lowest level of glucose (1.04 g $100g^{-1}$ FJ) as compared to control and all other treatments (Fig. 4.8B).

4.3.7.3. Sucrose

When averaged over ripening time, mean sucrose level was significantly ($P \leq 0.05$) higher (9.74 g $100g^{-1}$ FJ) in the 'Honey Fire' nectarine fruit which were coated with chitosan emulsion (1.5%) alone compared to control and all other treatments (Table 4.1). Mean concentration of sucrose was significantly ($P \leq 0.05$) lowest (7.85 g $100g^{-1}$ FJ) in the 'Honey Fire' nectarine fruit which were coated with OA (2.0 mM) alone as compared to all other treatments and control. When averaged over treatment, mean concentrations of sucrose decreased significantly in the 'Honey Fire' nectarine fruit from day three (9.89 g $100g^{-1}$ FJ) to seven after treatment (7.74 g $100g^{-1}$ FJ). The interaction between the treatments and the ripening period for sucrose concentration was found to be significant ($P \leq 0.05$) in 'Honey Fire' nectarine fruit. The levels of sucrose in ripe 'Bright Pearl' nectarine fruit did not differ significantly among different treatments and control (Fig. 4.8C). 'Bright Pearl' nectarine fruit coated with chitosan emulsion (1.5%) exhibited higher levels of sucrose (11.14 g $100g^{-1}$ FJ) followed by the fruit coated with chitosan emulsion loaded with 2.0 mM SA (10.19 g $100g^{-1}$ FJ) (Fig. 4.8C) and lowest in the fruit which were coated with 2.0 mM OA alone (7.72 g $100g^{-1}$ FJ).

4.3.7.4. Total sugars

When averaged over ripening time, mean concentrations of total sugars were higher (11.84 and 11.65 g $100g^{-1}$ FJ) in the ripe 'Honey Fire' nectarine fruit which were coated with chitosan emulsion (1.5%) alone and chitosan emulsion (1.5%) loaded with 2.0 mM OA respectively as compared with control and all other treatments (Table 4.1). Mean concentration of total sugars was lowest in the ripe 'Honey Fire' nectarine fruit which were treated with 2.0 mM OA alone compared to all other treatments. When averaged over all the treatments, mean concentration of total sugars decreased significantly from three to seven days after treatment (0.84-fold) in 'Honey Fire' nectarine fruit. The interaction between different treatments and the ripening period for concentrations of total sugars in 'Honey Fire' nectarine fruit was found to be significant. 'Bright Pearl' nectarine fruit coated with chitosan emulsion (1.5%) alone exhibited significantly ($P \leq 0.05$) higher levels of total sugars compared to all other treatments and control (Fig. 4.8D). The fruit coated with 2.0 mM OA showed lowest levels of total sugars (9.77g $100g^{-1}$ FJ) compared with all other treatments and control (Fig 4.8D).

Table 4.1. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of fructose, glucose, sucrose and total sugars in the juice of 'Honey Fire' cultivar of nectarine during fruit ripening.

Fructose (g $100g^{-1}$ FJ)				
Treatments	3 days	7 days	Mean(T)	LSD ($P \leq 0.05$)
Control	1.530	1.677	1.604 b	Treatments (T) = 0.16, Ripening period (RP) = 0.09, T x RP = NS
Chitosan	1.449	1.666	1.557 b	
Chitosan + salicylic acid	1.559	1.656	1.607 b	
Chitosan + oxalic acid	1.872	2.160	2.016 a	
Salicylic acid	1.615	1.726	1.671 b	
Oxalic acid	1.745	1.603	1.674 b	
Means (RP)	1.628 b	1.748 a		
Glucose (g $100g^{-1}$ FJ)				
	3 days	7 days	Mean(T)	LSD ($P \leq 0.05$)
Control	0.338	0.621	0.480 b	Treatments (T) = 0.07, Ripening period (RP) = 0.04, T x RP = 0.10
Chitosan	0.526	0.556	0.541 b	
Chitosan + salicylic acid	0.464	0.540	0.502 b	
Chitosan + oxalic acid	0.622	0.615	0.619 a	
Salicylic acid	0.461	0.568	0.514 b	
Oxalic acid	0.425	0.525	0.475 b	
Means (RP)	0.473 b	0.571 a		
Sucrose (g $100g^{-1}$ FJ)				
	3 days	7 days	Mean(T)	LSD ($P \leq 0.05$)
Control	9.55	7.53	8.54 c	Treatments (T) = 0.47, Ripening period (RP) = 0.27, T x RP = 0.67
Chitosan	10.41	9.07	9.74 a	
Chitosan + salicylic acid	10.03	8.28	9.16 b	
Chitosan + oxalic acid	11.73	6.30	9.02 bc	
Salicylic acid	8.65	8.50	8.58 c	
Oxalic acid	8.95	6.74	7.85 d	
Means (RP)	9.89 a	7.74 b		
Total sugars (g $100g^{-1}$ FJ)				
	3 days	7 days	Mean(T)	LSD ($P \leq 0.05$)
Control	11.42	9.83	10.63 bc	Treatments (T) = 0.47, Ripening period (RP) = 0.27, T x RP = 0.66
Chitosan	12.38	11.29	11.84 a	
Chitosan + salicylic acid	12.05	10.47	11.27ab	
Chitosan + oxalic acid	14.22	9.08	11.65 a	
Salicylic acid	10.73	10.79	10.77 b	
Oxalic acid	11.12	8.87	10.00 c	
Means (RP)	11.99 a	10.06 b		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates, ten fruits per replication.

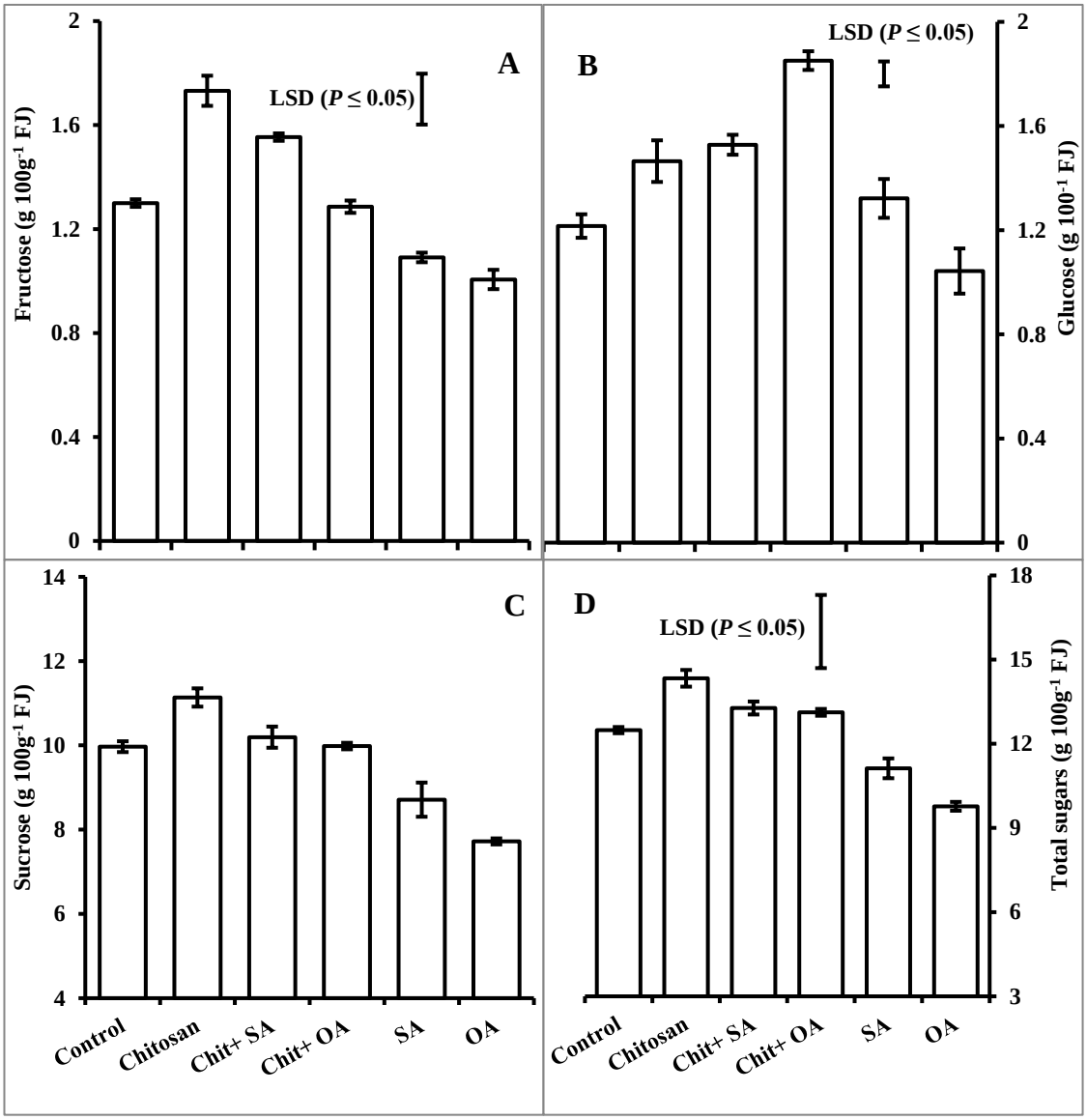


Figure 4.8. A-D. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of (A) fructose, (B) glucose, (C) sucrose and (D) total sugars in the juice of 'Bright Pearl' cultivar of nectarine at ripe stage. Vertical bars represent SE, n = four replicates, ten fruit in each replication.

4.3.8. Organic acids

Five organic acids were detected in nectarine fruit namely citric acid, malic acid, fumeric acid, tartaric acid and succinic acid. Citric acid is a major organic acid in 'Honey Fire' nectarine fruit. Meanwhile, malic acid is a predominant organic acid in 'Bright Pearl' nectarine fruit (Table 4.2 and Fig. 4.9).

4.3.8.1. Citric acid

The fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM oxalic acid showed significantly ($P \leq 0.05$) highest mean levels of citric acid (0.35 g $100g^{-1}$ FJ), when averaged over ripening time, compared to control (0.23 g $100g^{-1}$ FJ) and all other treatments in 'Honey Fire' nectarine fruit at ambient condition (Table 4.2). When averaged over all treatments, mean level of citric acid increased significantly (1.20-fold) from three to seven days after treatment in 'Honey Fire' nectarine. The interaction between different treatments and ripening period for citric acid was found to be significant in 'Honey Fire' nectarine fruit. 'Bright Pearl' nectarine fruit coated with 2.0 mM SA alone showed significantly higher concentration of citric acid (0.30 g $100g^{-1}$ FJ) in ripe fruit compared to the control (0.18 g $100g^{-1}$ FJ) and all other treatments (Fig. 4.9A).

4.3.8.2. Fumeric acid

When averaged over ripening time, mean levels of fumeric acid in 'Honey Fire' nectarine fruit did not differ significantly among different treatments and control (Table 4.2). When averaged over different treatments, mean levels of fumeric acid reduced (0.94-fold) significantly from three to seven days after treatments in 'Honey Fire' nectarine fruit. The interaction between different treatments and ripening period for levels of fumeric acid in 'Honey Fire' nectarine fruit was found to be significant. 'Bright Pearl' nectarine fruit coated with chitosan emulsion (1.5%) alone showed significantly highest concentration of fumeric acid (7.65 mg $100g^{-1}$ FJ) at ripe stage followed by chitosan emulsion (1.5%) loaded with 2.0 mM SA (5.00 mg $100g^{-1}$ FJ) (Fig. 4.9B).

4.3.8.3. Malic acid

When averaged over ripening time, 'Honey Fire' nectarine fruit which were coated with 2.0 mM SA or 2.0 mM OA resulted in significantly ($P \leq 0.05$) highest levels of malic acid (126.9 and 128.20 mg $100g^{-1}$ FJ respectively) as compared to the control and all other treatments (Table 4.2). When averaged over treatments, mean level of malic acid decreased (0.95-fold) significantly from three to seven days after treatment in 'Honey Fire' nectarine fruit. The interaction between different treatments and the ripening period for levels of malic acid in 'Honey Fire' nectarine fruit was found to be significant. Ripe 'Bright Pearl' nectarine fruit which were coated with chitosan emulsion (1.5%) loaded with 2.0 mM OA or 2.0 mM SA or 2.0

mM OA alone exhibited significantly lower levels of malic acid (187.4, 111.8 and 128.0 mg $100g^{-1}$ FJ) compared to those coated with chitosan emulsion (1.5%) and loaded with 2.0 mM SA or uncoated fruit (Fig. 4.9C).

4.3.8.4. Succinic acid

When averaged over ripening time, mean concentration of succinic acid in 'Honey Fire' nectarine fruit was significantly ($P \leq 0.05$) higher (2.76 mg $100g^{-1}$ FJ) in fruit which were coated with chitosan emulsion (1.5%) loaded with OA compared to control and all other treatments (Table 4.2). When averaged over ripening time, mean concentration of succinic acid increased (1.08-fold) significantly from three to seven days after treatment in 'Honey Fire' nectarine fruit. A significant interaction for levels of succinic acid between different treatments and ripening time was recorded in 'Honey Fire' nectarine fruit. There was no significant effect of different treatments on the concentration of succinic acid in 'Bright Pearl' nectarine fruit; however chitosan treated fruit showed the highest levels of succinic acid (4.09 mg $100g^{-1}$ FJ) in ripe 'Bright Pearl' nectarine fruit (Fig. 4.9D).

4.3.8.5. Tartaric acid

When averaged over ripening time, mean concentrations of tartaric acid were significantly ($P \leq 0.05$) highest (23.0 and 22.75 mg $100g^{-1}$ FJ) in 'Honey Fire' nectarine fruit which were coated with chitosan emulsion (1.5%) loaded with 2.0 mM SA and uncoated fruit respectively as compared to all other treatments (Table 4.2). When averaged over different treatments, mean level of tartaric acid increased (1.22-fold) significantly from three to seven days after treatment in 'Honey Fire' nectarine fruit. The interaction between different treatments and ripening period was found to be significant for levels of tartaric acid in 'Honey Fire' nectarine fruit. The level of tartaric acid was significantly ($P \leq 0.05$) highest (51.67 mg $100g^{-1}$ FJ) in 'Bright Pearl' nectarine fruit coated with chitosan emulsion (1.5%) alone as compared to all other treatments and control (Fig. 4.9E).

4.3.8.6. Total organic acids

When averaged over ripening time, 'Honey Fire' nectarine fruit treated with 2.0 mM OA exhibited significantly ($P \leq 0.05$) highest level of total organic acids (1.79 g $100g^{-1}$ FJ) compared to control and all other treatments (Table 4.2). When averaged over different treatments, mean levels of total organic acids did not differ

significantly from three to seven days after treatment in 'Honey Fire' nectarine fruit (Table 4.2). 'Bright pearl' nectarine fruit exhibited significantly highest levels of total organic acids (1.20 g $100g^{-1}$ FJ) when coated with chitosan emulsion (1.5%) alone compared with control and all other treatments (Fig. 4.9F).

4.3.9. Vitamin C

Higher concentration of vitamin C in 'Honey Fire' nectarine fruit (13.29 mg 100 g^{-1} FW) was recorded three days after the treatment of SA (2.0 mM) alone followed by the treatment of 1.5% chitosan emulsion loaded with SA (11.93 mg 100 g^{-1} FW) and chitosan alone (10.41 mg 100 g^{-1} FW) (Fig. 4.10A). A significant increase of vitamin C concentration (1.24-fold) was noted from the third to seventh day after treatment in the 'Honey Fire' nectarine fruit which was coated with chitosan emulsion loaded with SA. Meanwhile, 'Bright Pearl' nectarine fruit coated with chitosan emulsion loaded with SA showed significantly lowest concentration of vitamin C (0.61-fold) than the control fruit (16.56 mg 100 g^{-1} FW) (Fig. 4.10B). All the treatments have reduced the levels of vitamin C in 'Bright Pearl' nectarine fruit compared to the control (Fig. 4.10B)

Table 4.2. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of citric acid, malic acid, tartaric acid, fumeric acid, succinic acid and total organic acids in the juice of 'Honey Fire' cultivar of nectarine during ripening period.

Citric acid (g $100g^{-1}$ FJ)				
Treatments	3 days	7 days	Mean(T)	LSD ($P \leq 0.05$)
Control	0.166	0.301	0.234 d	Treatments (T) = 0.014, Ripening period (RP) = 0.082, T x RP = 0.020
Chitosan	0.223	0.340	0.282 b	
Chitosan + salicylic acid	0.264	0.297	0.281 b	
Chitosan + oxalic acid	0.333	0.359	0.346 a	
Salicylic acid	0.250	0.280	0.265 c	
Oxalic acid	0.292	0.263	0.278 bc	
Means (RP)	0.255 b	0.307 a		
	Malic acid (mg $100g^{-1}$ FJ)			LSD ($P \leq 0.05$)
Control	127.0	110.6	118.8 b	Treatments (T) = 2.39, Ripening period (RP) = 1.38, T x RP = 3.38
Chitosan	108.8	112.9	110.8 d	
Chitosan + salicylic acid	102.2	124.8	113.5 c	
Chitosan + oxalic acid	121.2	102.1	111.6 cd	
Salicylic acid	132.2	121.7	126.9 a	
Oxalic acid	138.1	118.4	128.2 a	

Means (RP)	121.6 a	115.1 b		
	Tartaric acid (mg 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	18.70	26.70	22.75 a	Treatments (T) = 2.86, Ripening period (RP) = 1.65, T x RP = 4.05
Chitosan	18.00	18.50	18.25 b	
Chitosan + salicylic acid	18.00	28.00	23.00 a	
Chitosan + oxalic acid	18.00	19.70	18.87 b	
Salicylic acid	18.00	19.50	18.75 b	
Oxalic acid	18.00	19.70	18.87 b	
Means (RP)	18.1 b	22.0 a		
	Fumeric acid (mg 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	22.50	11.83	17.16	Treatments (T) = NS, Ripening period (RP) = 0.46, T x RP = 1.15
Chitosan	17.13	17.58	17.35	
Chitosan + salicylic acid	16.85	17.08	16.96	
Chitosan + oxalic acid	15.88	16.60	16.24	
Salicylic acid	16.15	18.03	17.09	
Oxalic acid	16.45	17.45	16.95	
Means (RP)	17.49 a	16.43 b		
	Succinic acid (mg 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	2.215 cd	2.576 abc	2.39 b	Treatments (T) = 0.12, Ripening period (RP) = 0.07, T x RP = 0.18
Chitosan	2.127 d	2.669 ab	2.40 b	
Chitosan + salicylic acid	2.403	2.346	2.37 b	
Chitosan + oxalic acid	2.681	2.837	2.76 a	
Salicylic acid	2.013	2.159	2.09d	
Oxalic acid	2.262	2.217	2.24 c	
Means (RP)	2.284 b	2.467 a		
	Total organic acids (g 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	1.662	1.669	1.67 c	Treatments (T) = 0.03, Ripening period (RP) = NS, T x RP = 0.048
Chitosan	1.527	1.740	1.63 c	
Chitosan + salicylic acid	1.531	1.784	1.66 c	
Chitosan + oxalic acid	1.816	1.667	1.74 b	
Salicylic acid	1.776	1.717	1.75 b	
Oxalic acid	1.904	1.672	1.79 a	
Means (RP)	1.703	1.708		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates, ten fruits per replication.

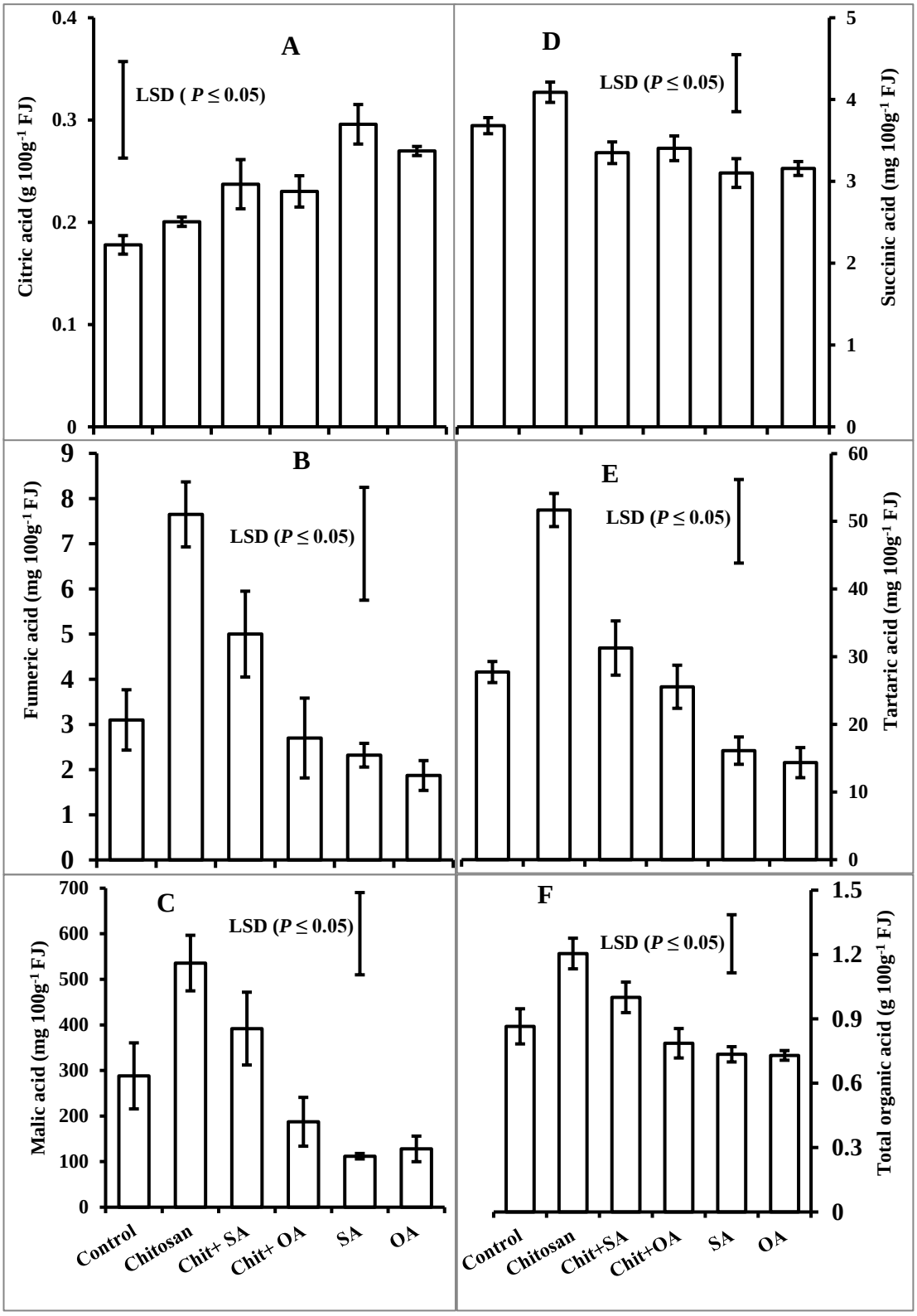


Figure 4.9. A-F. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of (A) citric acid, (B) fumeric acid, (C) malic acid, (D) succinic acid, (E) tartaric acid and (F) total organic acids in the juice of 'Bright Pearl' cultivar of nectarine during ripening period. Vertical bars represent SE, n = four replicates, ten fruit in each replication.

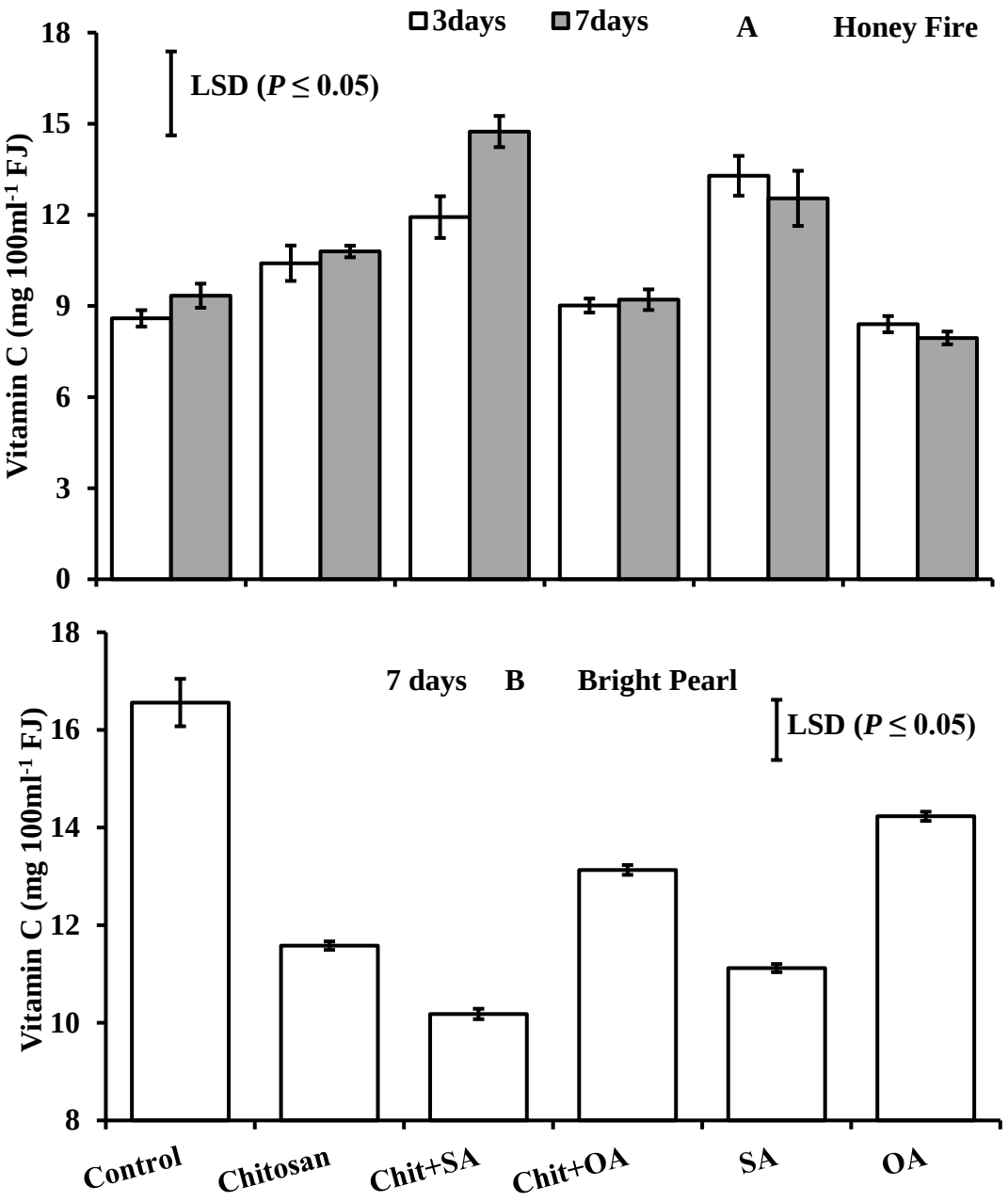


Figure 4.10. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA acid on levels of vitamin C during fruit ripening period in (A) Honey Fire and at ripe stage in (B) Bright Pearl cultivar of nectarine. Vertical bars represent SE, n = four replicates, ten fruit in each replication.

4.3.10. Total antioxidants

After three days of treatment, higher level of total antioxidants (46.83 µM Trolox 100 ml^{-1} FJ) was recorded in the 'Honey Fire' nectarine fruit coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) followed by the fruit coated with SA (2.0 mM) alone (45.14 µM Trolox 100 ml^{-1} FJ) (Fig. 4.11A). The changes in the level of total antioxidants in 'Honey Fire' nectarine fruit from the third to seventh day after treatment were non-significant. However, a slight increase was observed in control (1.03-fold), chitosan (1.01-fold), and SA (1.04-fold) coated 'Honey Fire' nectarine fruit after seven days of treatment. In 'Bright Pearl' nectarine fruit, higher concentration of total antioxidants was recorded in the fruit coated with SA (1.08-fold) followed by chitosan emulsion loaded with SA (1.06-fold) and chitosan emulsion alone (1.05-fold) than the control fruit (44.57 µM Trolox 100 ml^{-1} FJ) (Fig. 4.11B). The lowest level of antioxidants (42.78 µM Trolox 100 ml^{-1} FJ) was recorded in 'Bright Pearl' nectarine fruit which were coated with OA alone (Fig. 4.11B).

4.3.11. Disease incidence

'Bright Pearl' cultivar nectarine fruit coated with emulsion of chitosan (1.5%) loaded with 2.0 mM SA exhibited lowest percentage disease incidence (2.5%) seven days after ripening at ambient temperature, followed by the fruit coated with chitosan emulsion, SA alone and chitosan emulsion loaded with OA (5.0%, 7.5% and 10.0% respectively). Untreated fruit exhibited significantly ($P \leq 0.05$) highest percentage disease incidence (35.0%) as compared to all other treatments except 2.0 mM OA alone (22.5%), 7 days after ripening at ambient temperature (Fig. 4.12).

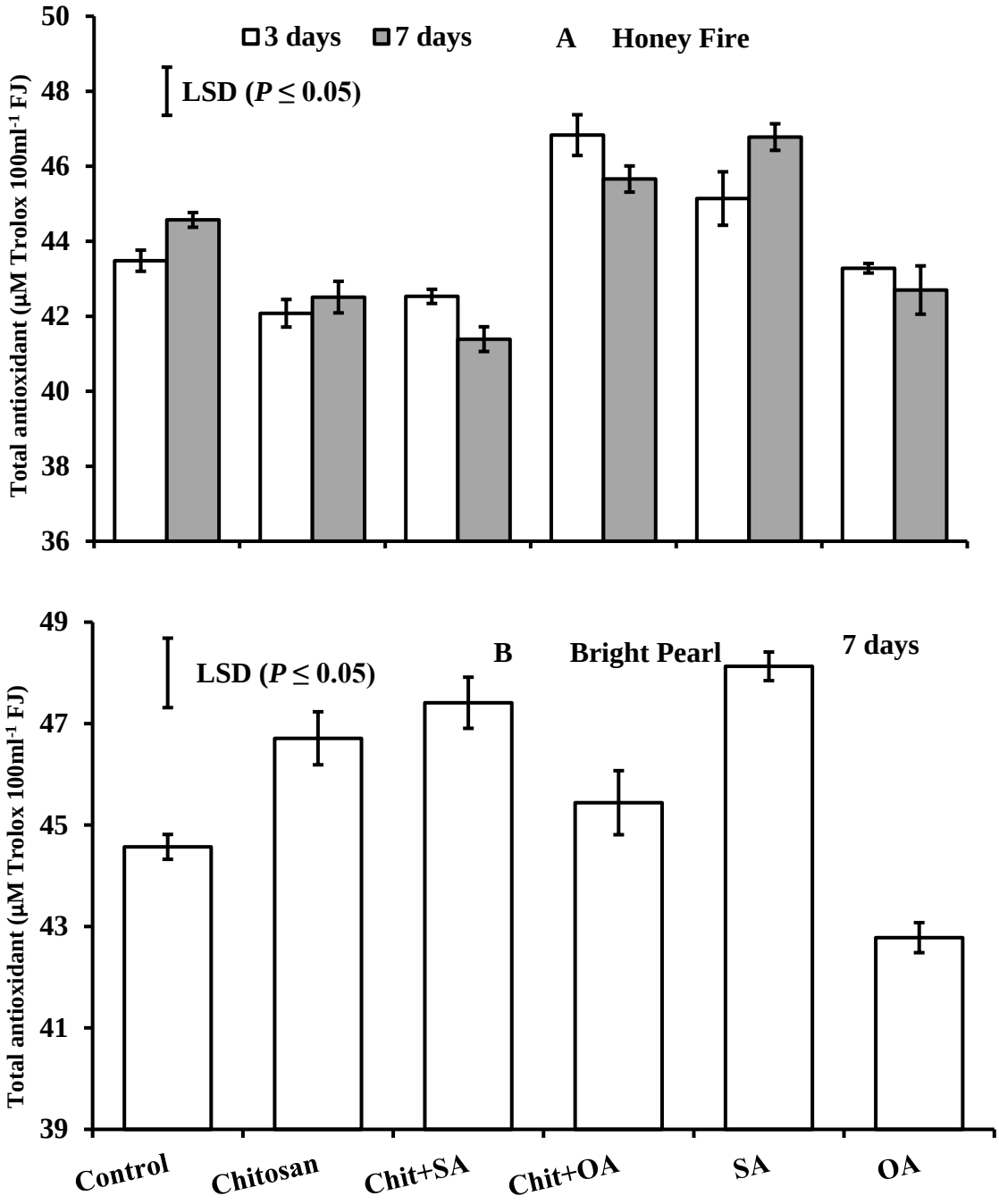


Figure 4.11. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA acid on levels of total antioxidants during fruit ripening period in (A) Honey Fire and at ripe stage in (B) Bright Pearl cultivar of nectarine. Vertical bars represent SE, n = four replicates, ten fruit in each replication.

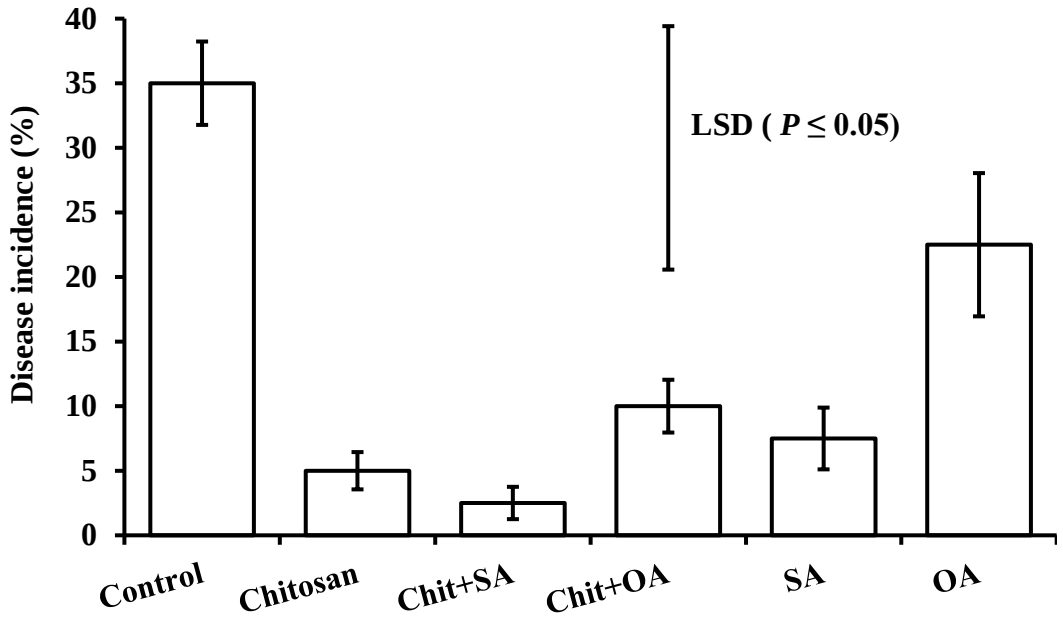


Figure 4.12. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on disease incidence during fruit ripening period in Bright Pearl cultivar of nectarine. Vertical bars represent SE, n = four replicates, ten fruit in each replication.

4.4. Discussion

Edible coatings are known to modify gaseous composition around the surface of fruits and vegetables, reduce loss of moisture and postharvest decay, maintain appearance, extend shelf life and maintain fruit quality to varying degrees of success during postharvest handling (Baldwin et al., 1995; Petersen et al., 1999; Romanazzi et al., 2003; Cha and Chinnan, 2004; Valverde et al., 2005). The edible coating materials (alginate, cellulose, chitosan, chitin, lipids, mucilage, milk protein, starch, wax, and zein) create an atmosphere similar to modified atmosphere packaging (MAP) and also show natural biocide activity (Cha and Chinnan, 2004). Better maintenance of fruit quality was observed by using chitosan on plum fruit (Bal, 2013), strawberry (Vu et al., 2011), peach (Li and Yu, 2001), papaya (Asgar et al., 2011) and nectarine (Giacalone and Chiabrando, 2015). Some studies reported that SA postharvest dip treatment improved storability, prolonged shelf life and lowered fruit decay in peach (Khademi and Ershadi, 2013) and plum (Davarynjad et al., 2013). Delayed fruit ripening, decreased ethylene production, maintenance of fruit quality and disease resistance have been reported for various fruits when OA (1-5 mM) is applied as a dip treatment to fruits such as peach (Zheng et al., 2007a),

mango (Zheng et al., 2007) and plum (Wu et al., 2011). However, no information is available on the effects of postharvest application of chitosan emulsion loaded with SA or OA on ethylene production, in modulating fruit ripening and quality of white flesh nectarine cultivar 'Honey Fire' and 'Bright Pearl' at ambient conditions. The results obtained from this study have been discussed in light of the previous observations by other researchers.

4.4.1. Ethylene production

The treatment of chitosan (1.5%) emulsion alone and loaded with SA (2.0 mM) treatment significantly ($P \leq 0.05$) suppressed the mean climacteric ethylene production (0.39-fold) during ripening 'Honey Fire' and 'Bright Pearl' nectarine fruit respectively (Fig. 4.1A and B). Possibly, the chitosan coating suppressed endogenous ethylene production in the coated fruit by reducing the activities of key ethylene biosynthesis enzymes such as 1-amino-cyclopropane carboxylic acid synthase (ACS) and 1-amino-cyclopropane carboxylic acid oxidase (ACO) enzymes (Noh, 2005). Moreover, ethylene biosynthesis is also dependent on the presence of O_2 (Abeles et al., 1992) and chitosan coating prevents the entry of oxygen into the fruit which ultimately reduces the level of endogenous ethylene (Noh, 2005). Similarly, suppressed ethylene production in different fruits coated with chitosan has also been reported previously for fruits such as tomatoes, cucumbers and bell peppers (El Ghaouth et al., 1992b). However, the effect of edible coating on the production of ethylene in a particular fruit is dependent on genotypes, which has also been reported by Noh, (2005). This is reflected in the results of the current study by the differential response of the nectarine cultivars to different treatments (Fig. 4.1). Chitosan emulsion (1.5%) suppressed mean ethylene production during ripening in cultivars 'Honey Fire'. Meanwhile chitosan emulsion loaded with SA (2.0 mM) was most effective in suppressing mean ethylene production during ripening in 'Bright Pearl' nectarine (Fig. 4.1B) and may be due to genotypic differences in the cultivars. SA is known to reduce ethylene production by increasing the activities of ACC synthase and ACC oxidase (Zhang et al., 2003). Some researchers also reported reduced ethylene production and delayed softening of plum (Wu et al., 2011) and jujube (Wang et al., 2009) by treating them with OA (Fig 4.1 and 4.2). Earlier, the application of OA has been reported to reduce ethylene production by decreasing the activity of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) (Wu et al., 2011).

4.4.2. Weight loss

Loss of fruit weight is the result of metabolic activity such as respiration and evaporation of moisture through the skin to air. The rate of fruit weight loss depends on the water pressure gradient between the fruit tissue and the surrounding atmosphere that is influenced by storage temperature (Ghasemnezhad et al., 2010). The positive effect of chitosan coating in reducing the loss of nectarine fruit weight was recorded in the current study. The lowest weight loss was recorded in chitosan emulsion coated fruit in both 'Honey Fire' and 'Bright Pearl' nectarine (3.6% and 5.46% respectively) compared to the control (7.49% and 8.85% respectively) and all other treatments (Fig 4.3). Possibly, the edible chitosan coating emulsion may have acted as a barrier to moisture loss and may have closed small wounds on the fruit surface and thereby delaying dehydration (Ribeiro et al., 2007). Prevention of the loss of weight by using chitosan coatings has also been reported previously in tomato (El Ghaouth et al., 1992b), longan fruit (Jiang and Li, 2001), banana and mango (Kittur et al., 2001), strawberries (Ribeiro et al., 2007) and plum (Bal, 2013). However, Ghasemnezhad et al. (2010) also reported that higher chitosan concentration may have increased anaerobic respiration followed by higher fruit weight loss. A combination of chitosan with other components may in the current study have resulted in higher weight loss; eg. in combined treatment of chitosan and SA (9.9%) in 'Honey Fire' nectarine fruit. Increase in weight loss of star fruit has been previously reported from the combined treatment of chitosan and stearin (C:S=2:1 and C:S=3:1) (Nurul Hanani et al., 2012). An edible coating comprising of chitosan is hydrophilic and acts as a gas barrier, whilst stearin is hydrophobic which demonstrated moisture barrier properties (Zaki et al., 2012). The variation in the fruit weight loss due to different treatments differs in both cultivars of nectarines may be ascribed to their genotypic differences between both cultivars but the exact mechanism is yet to be investigated.

4.4.3. Firmness

Higher firmness was recorded in the fruit coated with the chitosan emulsion (1.5%) loaded with 2.0 mM SA (2.20-fold) and chitosan alone (1.88-fold) than the control 'Honey Fire' nectarine fruit on the third day after treatment. These treatments also showed higher firmness in ripe 'Honey Fire' nectarine fruit (14.19 and 11.79 N respectively) on the seventh day after treatments (Fig 4.4). Fruit softening in

nectarine is related to the increased activities of cell wall-modifying enzymes such as polygalacturonase and pectin esterase (Manganaris et al., 2005b). The combined treatment of chitosan alone and chitosan loaded with SA suppresses the ethylene production in both 'Honey Fire' and 'Bright Pearl' nectarine fruit and the reduction in ethylene production may possibly have retarded the activities of fruit softening enzymes. Ethylene plays an important role in softening of fruits by regulating the activities of softening enzymes (PE, EGase, exo-PG and endo-PG) as reported previously by Khan and Singh (2007a). However, from the results of the current study, the firmness of nectarine fruit is a genotype dependent attribute since a higher level of firmness was observed in ripe 'Bright Pearl' nectarine fruit (23.58 N) than the 'Honey fire' nectarine fruit (14.19 N) treated with the combination of chitosan and SA.

4.4.4. SSC, TA and SSC:TA ratio

The edible coating with chitosan showed a reduction of SSC and TA value in nectarine compared to the control hence demonstrating a slowing down of the senescence process (Asgar et al., 2011; Chiabrando and Giacalone, 2013). Higher level of SSC was observed in the fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM SA and the higher TA was noted in the fruit coated with the chitosan emulsion loaded with 2.0 mM OA and OA alone after seven days of treatment in the 'Honey Fire' nectarine fruit (Fig. 4.5 and 4.6). Higher SSC:TA ratio in 'Honey Fire' and 'Bright Pearl' nectarine fruit was recorded in the fruit coated with the chitosan emulsion alone. Better maintenance of acidity in chitosan coated peaches has also reported by Li and Yu (2001) and Maftoonazad et al. (2008). Han et al. (2004) also reported that the chitosan coating slows down the ripening and prevents loss of titratable acidity in raspberry and strawberry fruit. On the contrary, the highest SSC and TA value in ripe 'Bright Pearl' nectarine fruit was recorded in the fruit which were coated with OA treatments which suggest that the effect of edible coating is genotype dependant in nectarines. Various coating treatments have influenced SSC, TA and their ratio in nectarine fruit possibly through regulation of ethylene production and consequent modulation of the ripening process. However, its exact mechanism warrants investigation.

4.4.5. Organic acids and sugars

The major organic acids in *Prunus* fruits are citric acid and malic acid (Le Dantec et al., 2010; Wu et al., 2011). Fumeric acid, tartaric acid and succinic acid have also been identified in different *Prunus* fruits (Flores et al., 2012). From the current study it was also observed that the dominant organic acids in the 'Honey Fire' and 'Bright Pearl' nectarine fruits were citric acid and malic acid followed by fumeric acid, tartaric acid and succinic acid. The dominant sugar in these fruit was sucrose followed by fructose and glucose. Previous research has also reported sucrose, fructose and glucose as the major sugar components in stone fruits along with some other monosaccharides and their derivatives such as stachyose (Sozzi, 2004), sorbitol (Cantín et al., 2009), raffinose (Ledbetter et al., 2006), rhamnose (Kovács and Németh-Szerdahelyi, 2002), arabinose, galactose, and xylose (Gross and Sams, 1984). Fruits accumulate organic acids at the early stage of development which is reflected in their acidic taste (Shiratake and Martinoia, 2007). Furthermore, at the maturation and ripening stage sugars accumulate in the cell vacuoles with a simultaneous decrease in organic acids (Yamaki, 1984; Echeverria and Burns, 1989). Sugars and the organic acids profiles and their inter-conversion vary depending on the species of stone fruit (Bae et al., 2014). Similarly in the present study, comparatively higher concentration of total organic acids was noted in the 'Honey Fire' nectarine than the 'Bright Pearl' nectarine fruit (Table 4.1 and 4.2, Fig. 4.8 and 4.9).

In the current study, mean level of citric acid and succinic acid increased significantly from three to seven days after treatment in 'Honey Fire' nectarine. However, the 'Honey Fire' nectarine fruit treated with chitosan emulsion loaded with OA showed significantly highest mean levels of citric acid which signifies the effect of this coating treatment in reducing the metabolic activities and retardation of the ripening process (Jitareerat et al., 2007) which ultimately slows down the reduction of citric acid level in the fruit. Similarly, highest mean level of malic acid was recorded in SA and OA treated 'Honey Fire' nectarine fruit which was the reverse in 'Bright Pearl' nectarine fruit. Highest concentration of citric acid was recorded in SA treated 'Bright Pearl' nectarine fruit (Fig 4.9A) reflecting the genotypic variations among the nectarine cultivars (Wu et al., 2003). Palma et al. (2015) reported no significant effect of edible coatings on the changes of citric acid and malic acid in

cactus pear fruit during storage. Different coatings tested in the experiment did not affect the levels of fumeric acid during ripening of 'Honey Fire' nectarine fruit as recorded in Table 4.2. However, the treatments showed significant effect on the concentration of fumeric acid in ripe 'Bright Pearl' nectarine fruit where the highest value was recorded in chitosan coated fruit (Fig 4.9B). A significant decrease in the mean level of malic acid was observed from three to seven days after treatment in 'Honey Fire' nectarine fruit (0.95-fold) and can possibly be ascribed to the increased activity of malate dehydrogenase (MDH) enzyme. Earlier, Yong-Hong et al. (2007) reported a significant correlation between malate dehydrogenase (MDH) enzyme activity and fruit malic acid content with the activity of malic enzyme MDH increasing in the late period of fruit development whereby decreasing the content of malic acid in fruit.

The concentration of total sugars in the fruit is known to increase with the advancement of fruit ripening (Abbasi et al., 2009). Unripe fruit accumulate starch which converts into sugars by amylase enzyme during the ripening period (Tareen, 2011). Mean highest concentration of total sugars was observed in the 'Honey Fire' nectarine fruit coated with chitosan alone as compared to the other treatments (Table 4.1). The concentration of total sugars significantly increased in all treatments during storage except OA coated fruit of both 'Honey Fire' and 'Bright Pearl' nectarine fruit (Fig 4.8) where the production of ethylene, loss of firmness and loss of weight were also considerably high and these metabolic functions may have utilized the sugars in OA-coated fruit (Gul et al., 1990). The concentration of total sugars decreased significantly from three to seven days after treatment in 'Honey Fire' nectarine fruit and the highest reduction was observed in fruit coated with chitosan emulsion loaded with OA. Youssef et al. (2002) reported that the concentration of reducing sugars (glucose and fructose) remains higher in coated mango fruit during storage due to slower ripening processes which is in agreement with the observations of the current study where most of the coating treatments also showed higher concentration of reducing sugars along with lower rate of metabolic activities (Table 4.1 and Fig. 4.8). However, Palma et al. (2015) reported no significant effect of edible coatings on the changes of sugars in cactus pear fruit during storage which was also reflected in the current study by the no significant differences between the control and most of the coating treatments in respect of the mean levels of total sugars in 'Bright Pearl'

nectarine fruit (Fig 4.9). Sucrose is a major sugar component in nectarine fruit and its concentration increases in ripe nectarine fruit due to the higher activity of sucrose-phosphate synthase (Hubbard et al., 1991) and the coating treatments may have enhanced the activity of sucrose-phosphate synthase and is yet to be investigated. The response of the nectarine fruit to the coating treatments is also dependent on the genotype which is reflected in the present study by the differential concentration of individual sugars in 'Honey Fire' and 'Bright Pearl' nectarine fruit in respect of different coating treatments. This interaction between genotype and treatment effects was also reported by Wu et al. (2003).

4.4.6. Vitamin C

Highest concentration of vitamin C was found in the ripe 'Honey Fire' nectarine fruit after seven days of treatment with the combination of chitosan and SA treatment. Ruoyi et al. (2005) reported that the combination of chitosan coating with $CaCl_2$ inhibits ascorbic acid oxidase activity which helps to maintain a relatively higher level of vitamin C in 'Zhonghuashoutao' peach fruit. Higher levels of vitamin C in mango fruit coated with chitosan have been attributed to slow ripening rate of the treated fruit (Abbasi et al., 2009). Edible coatings reduce the permeability of O_2 and CO_2 (Srinivasa et al., 2002) and thereby hinders the oxidation of vitamin C by external factors (Sritananan et al., 2005). The present study results indicate that the effectiveness of the edible coating also depends on the fruit genotype since the lowest concentration of vitamin C was found in 'Bright Pearl' nectarine fruit treated with combined chitosan and SA (Fig. 4.10). Previous studies have shown that peach fruit treated by SA or OA alone showed higher level of vitamin C than untreated fruit (Tareen, 2011). This is similar to the findings of the current study where OA treated 'Bright Pearl' nectarine and SA treated 'Honey Fire' nectarine fruit showed higher levels of vitamin C than other treatments and control.

4.4.7. Total antioxidants

Increased total phenolics and antioxidant activity have been reported in chitosan (0.5%) coated apricot fruit during cold storage (Ghasemnezhad et al., 2010). However, significant effect of SA and OA alone in improving the antioxidative capacity of peach fruit (Zhang et al., 2007; Tareen, 2011; Khademi and Ershadi, 2013), papaya (Setha et al., 2000), mandarin (El-hilali et al., 2003), sugar apple fruit (Mo et al., 2008) and grapes (Asghari et al., 2013) have also been reported. These

observations are in agreement with the results obtained from the current study where higher levels of total antioxidants were found in the SA treated 'Honey Fire' and 'Bright Pearl' nectarine fruit than control and other treatments (Fig 4.11). Ripe 'Honey Fire' fruit which were coated with chitosan emulsion loaded with OA and 'Bright pearl' nectarine fruit coated with chitosan alone and SA alone showed significantly higher level of total antioxidants compared to the control and other treatments (Fig. 4.11). Previously, postharvest application of SA has been reported to improve total antioxidant activity in 'Elberta' peach fruit compared to untreated fruit (Khademi and Ershadi, 2013). The mechanism by which chitosan, SA and OA influence levels of total antioxidants in nectarine fruit is not known and warrants further investigation.

4.4.8. Disease incidence

'Bright Pearl' nectarine fruit coated with chitosan emulsion (1.5%), SA 2.0 mM alone or the chitosan emulsion loaded with the SA or OA exhibited significantly lower disease incidence compared to the control and the treatment of OA alone (22.5%) (Fig. 4.12). Chitosan application possibly may have inhibited the germination of fungal spores and mycelium growth on the fruit surface and /or may have activated the defence response of the fruit tissue through pathogen-related (PR) gene function, leading to expression of chitinases, chitosanase, β-glucanases, lignin and callose as reported by Zhang et al. (2011). Previous studies have indicated that chitosan could effectively inhibit postharvest diseases on various horticultural commodities (Romanazzi et al., 2003, Bal, 2013; Bautista-Banos et al., 2006). The beneficial effects in reducing disease incidence in fruit treated with SA may possibly be attributed to enhanced resistance of the nectarine fruit to various pathogens as reported earlier by Asghari and Aghdam (2010). Similarly, Khademi and Ershdi, (2013) and Asghari and Aghdam, (2010) also reported that SA treatment lowered plum fruit decay. Moreover, SA has been reported to decrease decay in peaches, pears, apples, nectarines and bananas (Mo et al., 2008). Chitosan emulsion loaded with the SA was the most effective treatment in reducing disease incidence in 'Bright Pearl' nectarine fruit as compared to the application of SA or chitosan alone. This effect may be ascribed to the combined beneficial effects of both chitosan and SA.

4.5. Conclusion

The edible coatings tested in the experiment showed significant effects on the physico-chemical and physiological properties of the 'Honey Fire' and 'Bright Pearl' nectarine fruit as compared to the control. The effect of these coating treatments on nectarine fruit ripening in ambient conditions appeared as dependent on fruit genotype. In conclusion, chitosan emulsion (1.5%) loaded with SA (2.0 mM) or OA (2.0 mM) were more effective compared to chitosan emulsion, SA and OA alone in maintaining most fruit quality parameters in 'Honey Fire' nectarine fruit. Meanwhile, chitosan emulsion (1.5%) alone was more effective in maintaining fruit quality compared to the control and chitosan emulsion loaded with SA or OA in 'Bright Pearl' nectarine fruit. The hypothesis that chitosan loaded with SA or OA is more effective than chitosan, SA and OA alone was proved by the findings of the present study in 'Honey Fire' nectarine, whilst in cv. Bright Pearl, the hypothesis was rejected. In future research, the response of large numbers of commercial nectarine cultivars to these coating treatments warrants investigation.

CHAPTER 5

Influence of chitosan emulsion, salicylic acid or oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid on cold storage life and fruit quality of nectarine (*Prunus persica* L. Batsch. cv nectarine)

Summary

Edible coatings such as chitosan, salicylic acid (SA) and oxalic acid (OA) have been tested to extend storage life and maintain quality in different fruits. However, the effect of chitosan emulsion loaded with SA or OA on various quality parameters of cold stored nectarine has not yet been examined. The current study was conducted to investigate the effects of chitosan, SA or OA alone and their combinations in regulating ripening processes and fruit quality in white flesh nectarine cultivar 'Bright Pearl' under cold conditions. When averaged over both cold storage periods, the fruit coated with chitosan (1.5%) emulsion loaded with SA (2.0 mM) suppressed mean ethylene production (0.48 µmol kg^{-1} h^{-1}) during ripening, and maintained quality of the ripe fruits which exhibited higher mean level of malic acid (937.8 mg 100 ml^{-1} FJ), fructose (2.23 g 100 ml^{-1} FJ) and vitamin C (11.59 mg 100 ml^{-1} FJ) in ripe 'Bright Pearl' nectarine fruit as compared to all other treatments. The treatment of chitosan emulsion alone maintained highest level of firmness (46.38 N), citric acid (194 mg 100 ml^{-1} FJ), fumaric acid (9.63 mg 100 ml^{-1} FJ), succinic acid (5.57 mg 100 ml^{-1} FJ), total organic acid (1.68 g 100 ml^{-1} FJ) and reduced disease incidence in ripe 'Bright Pearl' nectarine fruit. Highest concentration of antioxidants (45.53 µM Trolox 100 ml^{-1} FJ) and SSC:TA ratio (48.86%) was recorded in 'Bright Pearl' nectarine fruit treated with OA alone. Highest level of titratable acidity (TA) (0.36%), tartaric acid (20.11 mg 100 ml^{-1} FJ), sucrose (11.84 mg 100 ml^{-1} FJ), total sugars (14.64 g 100 ml^{-1} FJ) and reduced loss of weight (30.39%) were observed in SA treated 'Bright Pearl' nectarine fruit. In conclusion, chitosan emulsion, SA or OA alone were more effective in maintaining quality in four weeks cold stored fruit compared to chitosan emulsion loaded with SA or OA in 'Bright Pearl' nectarine fruit.

5.1. Introduction

Fruits play an important role in fulfilling nutritional requirements of humans. Fruits are considered a major natural source of many nutrients, such as carbohydrates, vitamins, proteins, minerals, fibre and dietary polyphenols (Wegmans, 2009). Furthermore, some fruits contain antioxidants such as flavonoids, phenolic acids, tannins and anthocyanins (Fu et al., 2011; Haminiuk, et al., 2012). These compounds inhibit the impacts of oxidative processes which cause some severe diseases of the human body, such as cancer, autoimmune diseases and multiple sclerosis (Kurosumi et al., 2007). Nectarine fruit is a rich source of different kinds of vitamins, minerals and anti-oxidative compounds (Gil et al., 2002). Nectarine is a climacteric fruit and has a short storage life ranging from 2 to 5 weeks (Ahmed et al., 2009). Nectarine fruit exhibits a higher rate of ethylene production and respiration, as well as significantly influences ripening and various fruit quality parameters during its ripening (Lill et al., 1989). Many approaches have been tested to delay ripening in nectarine fruit with limited success such as calcium application (Manganaris et al., 2006) and postharvest heat treatment (Obenland et al., 2005); 1-MCP fumigation (Liguori et al., 2004) and AVG (aminoethoxyvinylglycine) (Garner et al., 2001). Extending the storage period of nectarine fruit in controlled and modified atmosphere (Akbudak and Eris, 2004; Uthairatanakij et al., 2005) and in cold storage (Manganaris et al., 2005a) has also been reported. Chilling injury is a major physiological disorder in cold storage of nectarine fruit (Manganaris et al., 2005a).

Various edible coating constituents act as barriers to moisture and oxygen during postharvest handling and storage of fruit. Coatings are also used to improve the gloss of the commodity, extend shelf life and maintain fruit quality with varying success (Baldwin et al., 1995; Petersen et al., 1999; Cha and Chinnan, 2004; Valverde et al., 2005). Most of the edible coating materials act as natural biocides, are environmentally friendly and modify the atmosphere around the fruit (Cha and Chinnan, 2004). Although waxes are widely used as coating material they were not equally effective for all fruits (Cha and Chinnan, 2004). Lipid/hydrocolloid coatings have been reported to reduce loss of fruit firmness, crispness and juiciness in 8-week cold storage apples cv. Golden Delicious (Conforti and Totty, 2007). Meng et al. (2010) reported that chitosan coatings improve fruit quality and reduce postharvest

diseases in fruits such as pear and mango (Jitareerat et al., 2007), and strawberries (El Ghaouth et al., 1991).

Salicylic acid (SA) is a safe and natural endogenous phenolic compound in plants and is known to reduce postharvest losses of horticultural commodities (Asghari and Aghdam, 2010). SA is one of the main phenolic compounds that have been claimed to instigate various processes in plants including ethylene production, regulation of plant growth, development of sex polarization, seed germination and disease resistance (Babalar et al., 2007; Al-Qurashi and Awad, 2012). Khademi and Ershadi (2013) reported that postharvest dip application of SA (2.0 mM) gave improved fruit firmness and a reduction in weight loss and fruit decay on postharvest peach fruit, when stored at cold condition (0 ±1 °C) for 42 days. Furthermore, SA (0.8 mM) has been found to significantly decrease respiration rate, ethylene production and increase the activity of antioxidant enzymes in sugar apple fruit (*Amona squamosa* L.) (Mo et al., 2008). Moreover, dip application of SA (2.0 mM) has been reported to decrease the development of chilling injury compared to the control in pear fruit (Al-Qurashi and Awad, 2012). Recently, OA treatment has been applied for food preservation as a natural antioxidant (Zheng et al., 2007a). Zheng et al (2007b) also reported that dip application of OA delayed the loss of firmness and ripening as well as reduced ethylene production in mango fruit. Postharvest dip application of OA also lowered respiration rate and increased activities of antioxidant enzymes in peach fruit as compared with the control (Zheng et al., 2007a).

Earlier, the beneficial effects of chitosan coating (Giacalone and Chiabrando, 2015) and the exogenous application of SA (Khademi and Ershadi, 2013) and OA (Tareen, 2011) in extending cold storage life and maintenance of nectarine and peach fruit quality have been reported. However the effects of postharvest application of chitosan emulsion loaded with SA or OA on cold storage life and quality of nectarine fruit has yet to be investigated. It was surmised that chitosan loaded with SA or OA will be more effective in extending cold storage life and maintaining fruit quality of nectarine compared to chitosan coating, SA or OA alone. Therefore, this study aimed at elucidating the influence of chitosan coating, SA and OA alone or chitosan loaded with SA or OA in modulating cold storage life and quality of white flesh nectarine cultivar ‘Bright Pearl’.

5.2. Materials and methods

5.2.1. Plant material

Nectarine (*Prunus persica* L. Batch cv 'Bright Pearl') fruit were harvested on 8th of January 2014, at commercial maturity (SSC = 12.48%, fruit firmness = 71.28 N, ethylene production = 0.054 µmol kg^{-1} h^{-1}) from Casuarina Valley Orchard, Karragullen, Perth Hills (31° 57′S; 115° 50′E), Western Australia. The fruit used for this experiment were visually free from diseases and physiological disorders and of uniform size. The selected fruits were transported to the Horticulture Research Laboratory, Curtin University, Perth, WA, within one hour of harvest.

5.2.2. Treatments and experimental design

The mature fruit of nectarine cv. Bright Pearl were coated by spraying aqueous emulsion containing chitosan emulsion (1.5%), solution of SA (2.0 mM) or OA (2.0 mM) alone or the chitosan emulsion (1.5%) loaded with SA (2.0 mM) or OA (2.0 mM) and a surfactant known as Tween 20 (0.25%) in all the treatments except control. Uncoated fruits were kept as a control. After the treatments, the fruit were allowed to dry at room temperature, prior to transfering in cold storage (0 - 1° C and 95 ± 5% RH). Ethylene production, weight loss and disease incidence from the fruit were determined after four weeks of cold storage. Meanwhile, fruit firmness, soluble solids concentration (SSC), titratable acidity (TA), ratio of SSC and TA, total and individual sugars and organic acids, vitamin C and total antioxidants were determined after two and four weeks of cold storage. The experiment followed a completely randomized design (CRD) with four replications and 10 fruit in each replication.

5.2.3. Estimation of production of ethylene

Ethylene production was determined using an ETD 300 ethylene detector (Sensor sense B.V, Nijmegen, The Netherlands) by following the method of Pranamornkith et al. (2012) and detailed in Chapter 3 Section 3.4. Each sample ran for 20 min with an air flow rate of 4 L hr^{-1}. The ethylene production rate was calculated as µmol kg^{-1} h^{-1}.

5.2.4. Determination of loss of fruit weight

Fruit weight was estimated at the commencement and completion of the cold storage period. The weight loss was calculated as the percentage against the fruit weight at

the commencement of the cold storage period as detailed by Ahmad et al. (2013) and in Chapter 3, Section 3.6.

5.2.5. Determination of fruit firmness

The firmness of fruit pulp was estimated using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK) fitted with a horizontal square base table (15 cm × 15 cm) following the method of Singh et al. (2009) and also detailed in Chapter 3, Section 3.7. Fruit firmness was expressed as newtons (N).

5.2.6. Determination of SSC, TA and SSC:TA ratio

The SSC, TA and their ratio were estimated from the nectarine fruit juice extracted from the pulp of ten randomly selected fruit using a fruit juicer (Model JE8500, Sunbeam Corp. Ltd., Botany, Australia) as detailed in Chapter 3, Section 3.8.

5.2.7. Determination of individual sugars and organic acids

Individual sugars were determined by using an HPLC system (Waters 1525, Milford Corp., MA, USA) with a Bio-Rad Aminex® HPX-87C Fast Carbohydrate column (100 × 7.8 mm) and a refractive index (RI) detector (Water 2414, Milford Corp., MA, USA). Individual organic acids were separated using an HPLC system (Waters 1525, Milford Corp., MA, USA) with Bio-Rad Aminex® HPX-87H column (300 × 7.8 mm) (Bio-Rad Laboratories, Inc., Hercules, USA) and a dual wavelength absorbance detector (Waters 2487, Milford Corp., MA, USA). Breeze® 3.30 software (Waters, Milford Corp., MA, USA) was used to process the collected data. The detailed methods have been previously described in Chapter 3, Section 3.9.

5.2.8. Determination of vitamin C

Vitamin C concentrations were estimated by following the method detailed by Malik and Singh (2005) using a UV/VIS spectrophotometer (Jenway Spectrophotometer Model 6405, Dunmow, Essex, UK). Vitamin C concentration was expressed as mg vitamin C 100 ml^{-1} FJ equivalent of L-ascorbic acid. The detailed method has also been explained in Chapter 3, Section 3.10.

5.2.9. Determination of total antioxidants

Total antioxidants were determined employing the modified procedure of Brand-William et al. (1995) and Pham (2009) using a 6405 UV/VIS spectrophotometer (Model 6405, Dunmow, Essex, UK). A standard curve of 6-hydroxy-2, 5, 7, 8-

tetramethylchromane-2-carboxylic acid (Trolox) was used for calculating total antioxidants. Total antioxidants were expressed as µM trolox equivalent antioxidant activity (TEAC) 100 ml^{-1} FJ basis. The detailed method has been described earlier in Chapter 3, Section 3.11.

5.2.10. Determination of disease incidence

The disease incidence was determined by examining the fruit regularly and fruit was regarded as infected if a visible lesion was observed and expressed as a percentage and also explained in Chapter 3, Section 3.13.

5.2.11. Statistical analysis

The data on various parameters showing effects of different coating treatments and cold storage period of nectarine fruit were analysed using one-way or two-way analysis of variance (ANOVA), by employing GenStat 14th edition (Lawes Agricultural Trust, Rothamsted experimental station, UK). The effects of different coating treatments, cold storage period and their interactions on different parameters were assessed within ANOVA by using least significant differences (LSD). The LSD was calculated following significance F test at $P \leq 0.05$.

5.3. Results

5.3.1. Ethylene Production

The treatment of chitosan emulsion (1.5%) loaded with SA (2.0 mM), SA (2.0 mM) alone and the treatment of chitosan emulsion (1.5%) loaded with OA (2.0 mM) significantly ($P \leq 0.05$) suppressed ethylene production (0.48, 0.55 and 0.61-fold respectively) following four-week cold storage in 'Bright Pearl' nectarine fruit in comparison to the control fruit (0.98 µmol kg^{-1} h^{-1}) and other treatments (Fig. 5.1).

5.3.2. Weight loss

Least weight loss was recorded in fruit coated with SA (2.0 mM) alone (30.39%) as compared to the uncoated fruit and other treatments (Fig 5.2). The loss of weight was highest in the fruit that were coated with OA (2.0 mM) alone (36.96%) followed by control fruit (34.99%) and the treatment of chitosan (1.5%) emulsion loaded with OA (2.0 mM) (Fig. 5.2).

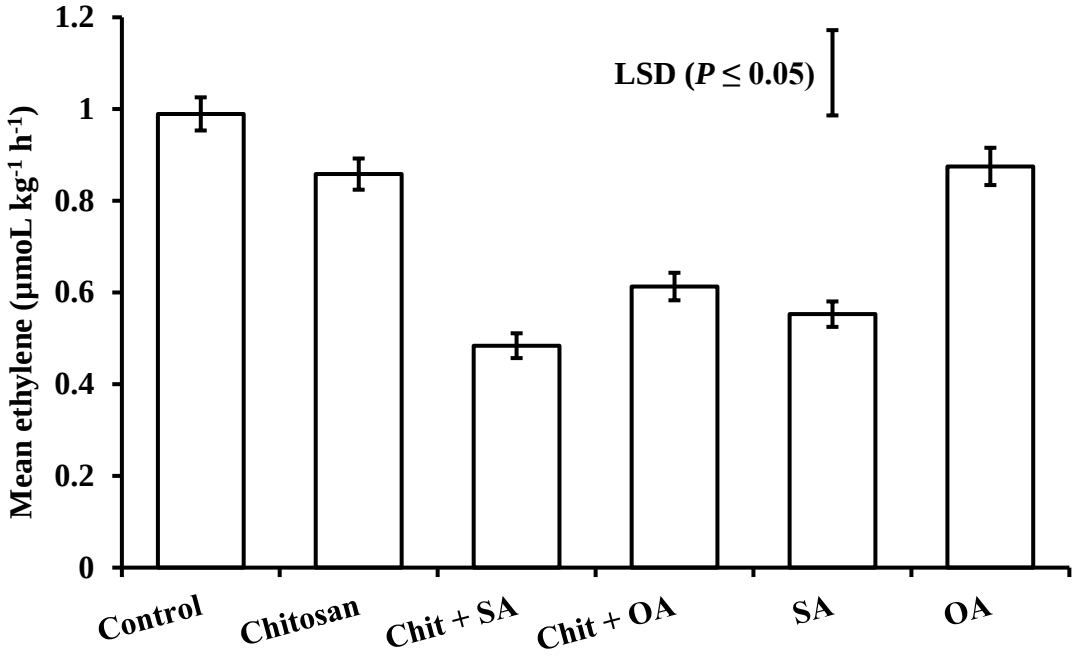


Figure 5.1. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on mean ethylene production in 'Bright Pearl' nectarine fruit following four weeks cold storage. Vertical bars represent SE, n = four replicates, two fruit in each replication.

5.3.3. Firmness

The fruit coated with chitosan emulsion or SA alone; and chitosan emulsion loaded with SA exhibited significantly higher fruit firmness (46.38N, 45.56N and 42.60N respectively) compared to all other treatments and control (Table 5.1). When averaged over cold storage period control fruit showed significantly lowest mean fruit firmness (32.40N) in comparison to all other treatments. When averaged over different treatments mean firmness was significantly lowest in the 4 weeks cold stored fruit (21.39N) than two weeks cold stored fruit (59.91N). The fruit coated with chitosan emulsion or SA alone; and chitosan emulsion loaded with SA exhibited higher fruit firmness in both two and four weeks cold storage. The interaction between different treatments and cold storage period was found to be significant for firmness in 'Bright Pearl' nectarine fruit (Table 5.1).

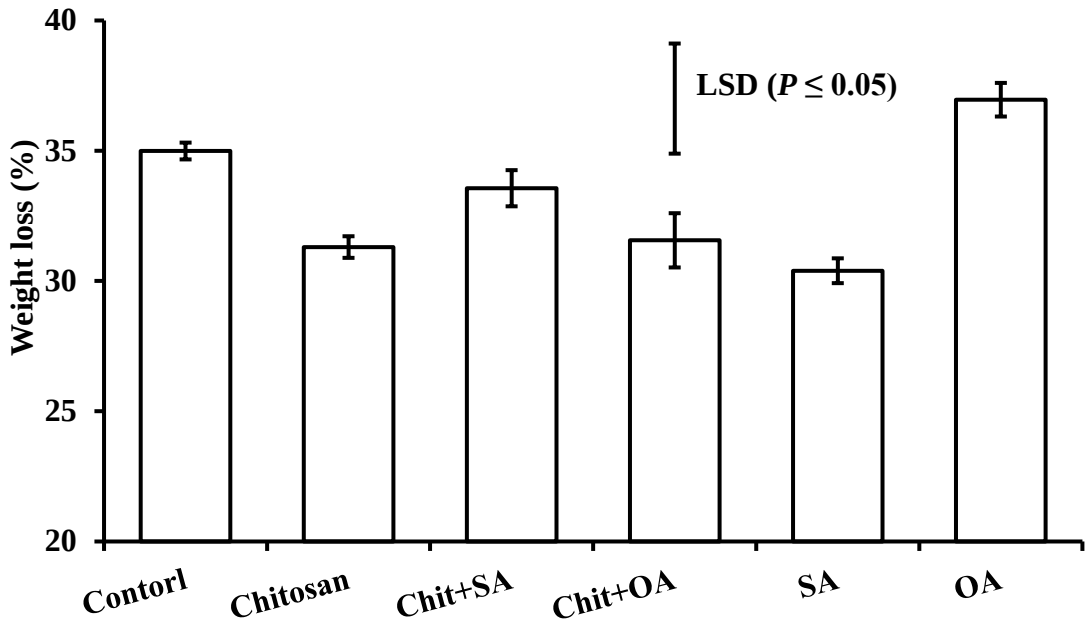


Figure 5.2. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on weight loss in 'Bright Pearl' nectarine fruit following four-week cold storage period. Vertical bars represent SE, n = four replicates, ten fruit in each replication.

5.3.4. Soluble solids concentration (SSC)

When averaged over two cold storage periods, mean SSC in 'Bright Pearl' nectarine was significantly higher (15.30%) in uncoated fruit compared to all other coating treatments (Table 5.1). Averaged over treatments, mean SCC was significantly higher (15.12%) in four weeks cold stored than two weeks cold stored (14.08%) in 'Bright Pearl' nectarine fruit (Table 5.1) The interaction between different coating treatments and the cold storage period for SSC was not significant (Table 5.1).

5.3.5. Titratable acidity (TA)

Fruit coated with OA alone resulted in significantly lowest level of mean acidity averaged over two cold storage periods compared to the control and all other coating treatments (Table 5.1). When averaged over different treatments, mean acidity levels was significantly lower following four weeks cold storage (0.318%) than two weeks storage (0.356%). The interaction between different treatments and cold storage periods was found to be significant for levels of total acidity in 'Bright Pearl' nectarine fruit. The coating treatment of chitosan emulsion (1.5%) loaded with SA (2.0 mM) showed highest level of total acidity (0.38%) compared to all other

treatments following two weeks cold storage. Fruit coated with OA alone showed significantly lowest levels of total acidity in 'Bright Pearl' nectarine fruit following two and four weeks of cold storage (0.31% and 0.29% respectively) as compared to all other treatments and control (Table 5.1).

5.3.6. SSC:TA ratio

When averaged over cold storage periods, 'Bright Pearl' nectarine fruit coated with OA (2 mM) resulted in significantly higher mean SSC:TA ratio (48.86) compared to control and all other treatments (Table 5.1). When averaged over treatments, mean SSC:TA ratio was significantly higher in four weeks cold stored fruit (47.68) than two weeks cold stored 'Bright Pearl' nectarine fruit (39.76). The interaction between different coating treatments and the cold storage period was found to be non-significant for SSC:TA ratio (Table 5.1).

5.3.7. Individual sugars

Sucrose was observed as the major sugar component in 'Bright Pearl' nectarine followed by fructose and glucose.

5.3.7.1. Fructose

When averaged over both cold storage periods mean levels of fructose were significantly highest (2.23 mg $100g^{-1}$ FJ) in the 'Bright Pearl' nectarine fruit coated with chitosan (1.5%) emulsion loaded with SA (2.0 mM) followed by fruit coated with chitosan (1.5%) emulsion alone (2.09 g $100g^{-1}$ FJ) and fruit coated with SA (2.0 mM) alone (2.00 g $100g^{-1}$ FJ) compared to control and other treatments (Table 5.2). When averaged over different treatments mean level of fructose was significantly higher (2.18 g $100g^{-1}$ FJ) in four weeks cold stored 'Bright Pearl' nectarine fruit than the two weeks stored fruit (1.56 g $100g^{-1}$ FJ). The interaction between different treatments and cold storage period was found to be significant for the levels of fructose in 'Bright Pearl' nectarine fruit (Table 5.2).

Table 5.1. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on firmness, SSC, TA and SSC: TA ratio following two and four weeks cold storage period in 'Bright Peal' nectarine fruit.

Firmness (N)				
	Cold storage period (weeks)			
Treatment	2	4	Mean(T)	LSD ($P \leq 0.05$)
Control	47.47	17.34	32.40 c	Treatments (T) = 3.69, Storage period (SP) = 2.13, T x SP = 5.21
Chitosan	68.32	24.43	46.38 a	
Chitosan + salicylic acid	63.54	21.65	42.60 a	
Chitosan + oxalic acid	56.36	20.26	38.31 b	
Salicylic acid	65.54	25.57	45.56 a	
Oxalic acid	58.21	19.07	38.64 b	
Means (SP)	59.91 a	21.39 b		
SSC (%)				
	2	4	Mean(T)	LSD ($P \leq 0.05$)
Control	14.67	15.92	15.30 a	Treatments (T) = 0.41, Storage period (SP) = 0.23, T x SP = NS
Chitosan	13.55	14.75	14.15 c	
Chitosan + salicylic acid	13.95	14.82	14.39 bc	
Chitosan + oxalic acid	14.07	14.90	14.49 bc	
Salicylic acid	14.30	15.15	14.73 b	
Oxalic acid	13.95	15.15	14.55 bc	
Means (SP)	14.08 b	15.12 a		
TA (%)				
	2	4	Mean(T)	LSD ($P \leq 0.05$)
Control	0.35	0.31	0.33 b	Treatments (T) = 0.01, Storage period (SP) = 0.008, T x SP = 0.02
Chitosan	0.37	0.34	0.35 a	
Chitosan + salicylic acid	0.38	0.32	0.35 a	
Chitosan + oxalic acid	0.36	0.30	0.33 b	
Salicylic acid	0.37	0.34	0.36 a	
Oxalic acid	0.31	0.29	0.30 c	
Means (SP)	0.356 a	0.318 b		
SSC: TA ratio				
	2	4	Mean(T)	LSD ($P \leq 0.05$)
Control	41.70	51.02	46.36 b	Treatments (T) = 2.42, Storage period (SP) = 1.40, T x SP = 3.42
Chitosan	36.89	43.74	40.32 d	
Chitosan + salicylic acid	36.97	45.71	41.34 d	
Chitosan + oxalic acid	38.85	48.96	43.90 c	
Salicylic acid	38.70	44.35	41.53 cd	
Oxalic acid	45.42	52.29	48.86 a	
Means (SP)	39.76 b	47.68 a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates, ten fruits per replication.

5.3.7.2. Glucose

All the coating treatments except OA (2.0 mM) alone resulted in significantly higher mean levels of glucose in Bright Pearl' nectarine fruit irrespective of the cold storage period compared to control (Table 5.2). 'Bright Pearl' nectarine fruit coated with OA (2.0 mM) alone showed significantly lowest mean levels of glucose (0.58 g $100g^{-1}$ FJ) compared to the control (0.66 g $100g^{-1}$ FJ) and all other treatments when averaged over both cold storage periods. There was a significant interaction between various treatments and cold storage period for levels of glucose in 'Bright Pearl' nectarine fruit (Table 5.2).

5.3.7.3. Sucrose

When averaged over both cold storage periods mean sucrose levels were significantly higher (11.84 g $100g^{-1}$ FJ) in the 'Bright Pearl' nectarine fruit coated with SA (2.0 mM) alone, followed by the fruit coated with chitosan (1.5%) emulsion loaded with 2.0 mM SA (11.48 g $100g^{-1}$ FJ) in comparison to control fruit (9.78 g $100g^{-1}$ FJ) and other treatments (Table 5.2). Bright Pearl' nectarine fruit coated with chitosan (1.5%) emulsion loaded with 2.0 mM OA showed significantly lowest mean levels of sucrose as compared to control and all other treatments. When averaged over different treatments, mean levels of sucrose in the fruit did not vary significantly between two and four weeks cold storage period. The interaction between different coating treatments and cold storage period was found to be significant for the levels of sucrose in 'Bright Pearl' nectarine fruit (Table 5.2).

5.3.7.4. Total sugars

'Bright Pearl' nectarine fruit coated with SA (2.0 mM) alone exhibited significantly higher mean concentrations of total sugars averaged over cold storage period (14.64 g $100g^{-1}$ FJ) followed by chitosan (1.5%) emulsion loaded with 2.0 mM SA (14.46 g $100g^{-1}$ FJ) compare to control (12.04 g $100g^{-1}$ FJ) and all other treatments (Table 5.2). Mean level of total sugars was significantly lowest in the fruit coated with chitosan (1.5%) emulsion loaded with 2.0 mM OA as compared to the control and all other treatments. Mean levels of total sugars when averaged over different treatments in the fruit did not vary significantly between two and four weeks cold storage period. The interaction between different coating treatments and cold storage period was found to be significant for the levels of total sugars in 'Bright Pearl' nectarine fruit (Table 5.2).

5.3.8. Organic acids

Five organic acids were detected and quantified in nectarine fruit such as citric acid, malic acid, fumaric acid, tartaric acid and succinic acid. Malic acid and citric acid are the major organic acids in 'Bright Pearl' nectarine fruit, followed by tartaric acid, fumaric acid and succinic acid (Table 5.3).

5.3.8.1. Citric acid

'Bright Pearl' nectarine fruit coated with chitosan (1.5%) emulsion alone and chitosan loaded with SA showed significantly highest mean levels of citric acid (194 mg $100g^{-1}$ FJ and 188 mg $100g^{-1}$ FJ respectively) compared to the control (174 mg $100g^{-1}$ FJ) and all other treatments when averaged over both cold storage periods (Table 5.3). When averaged over different treatments, mean levels of citric acid in four weeks cold stored Bright Pearl' nectarine fruit was significantly higher (182 mg $100g^{-1}$ FJ) than two week cold stored fruit (172 mg $100g^{-1}$ FJ). Four weeks cold storage of 'Bright Pearl' nectarine fruit coated with OA (2.0 mM) alone, SA (2.0 mM) alone or chitosan (1.5%) emulsion loaded with SA (2.0 mM) resulted in 1.15-fold, 1.08-fold and 1.07-fold increased levels of citric acid compared to the two week cold storage. The interaction between different treatments and cold storage period was found to be significant for the levels of citric acid in 'Bright Pearl' nectarine fruit (Table 5.3).

Table 5.2. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on fructose, glucose, sucrose and total sugars following two and four weeks cold storage period in 'Brigh Pearl' nectarine fruit.

Fructose (g 100 ml^{-1} FJ)				
	Cold storage period			
Treatment	2 weeks	4 weeks	Mean (T)	LSD ($P \leq 0.05$)
Control	1.39	1.82	1.60 b	Treatments (T) = 0.26, Storage period (SP) = 0.15, T x SP = 0.37
Chitosan	1.86	2.33	2.09 a	
Chitosan + salicylic acid	1.72	2.74	2.23 a	
Chitosan + oxalic acid	1.49	1.84	1.66 b	
Salicylic acid	1.51	2.50	2.00 a	
Oxalic acid	1.41	1.84	1.63 b	
Means (SP)	1.56 b	2.18 a		
Glucose (g 100 ml^{-1} FJ)				
Control	0.61	0.71	0.66 bc	Treatments (T) = 0.08, Storage period (SP) = NS, T x SP = 0.12
Chitosan	0.70	0.90	0.80 a	
Chitosan + salicylic acid	0.73	0.78	0.75 a	
Chitosan + oxalic acid	0.76	0.70	0.73 ab	
Salicylic acid	0.82	0.78	0.80 a	
Oxalic acid	0.66	0.49	0.58 c	
Means (SP)	0.71	0.73		
Sucrose (g 100 ml^{-1} FJ)				
Control	9.20	10.36	9.78 cd	Treatments (T) = 0.97, Storage period (SP) = NS, T x SP = 1.37
Chitosan	12.43	6.20	9.32 d	
Chitosan + salicylic acid	10.38	12.57	11.48 ab	
Chitosan + oxalic acid	8.93	6.07	7.50 e	
Salicylic acid	11.08	12.58	11.84 a	
Oxalic acid	8.67	12.40	10.54 bc	
Means (SP)	10.12	10.03		
Total sugars (g 100 ml^{-1} FJ)				
Control	11.19	12.89	12.04 b	Treatments (T) = 1.03, Storage period (SP) = NS, T x SP = 1.46
Chitosan	14.99	9.43	12.21 b	
Chitosan + salicylic acid	12.83	16.09	14.46 a	
Chitosan + oxalic acid	11.17	8.61	9.89 c	
Salicylic acid	13.41	15.86	14.64 a	
Oxalic acid	10.75	14.74	12.75 b	
Means (SP)	12.39	12.93		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates, ten fruits per replication.

5.3.8.2. Malic acid

When averaged over both cold storage periods mean levels of malic acid were significantly highest (937.8 mg $100g^{-1}$ FJ) in the 'Bright Pearl' nectarine fruit coated with chitosan (1.5%) emulsion loaded with SA (2.0 mM) followed by fruit coated with chitosan (1.5%) emulsion alone (907.7 mg $100g^{-1}$ FJ) compared to control and

all other treatments (Table 5.3). When averaged over different treatments mean level of citric acid was significantly lower in the 4 weeks cold stored fruit (751 mg 100g-1 FJ) than two weeks cold stored fruit (845 mg $100g^{-1}$ FJ). There was a significant interaction between various treatments and cold storage period for levels of malic acid in 'Bright Pearl' nectarine fruit (Table 5.3).

5.3.8.3. Tartaric acid

When averaged over both cold storage periods mean concentration of tartaric acid in 'Bright Pearl' nectarine fruit was significantly higher in the fruit treated with SA (2.0 mM) alone (21.11 mg $100g^{-1}$ FJ) followed by chitosan (1.5%) emulsion (19.66 mg $100g^{-1}$ FJ) alone compared to control (12.06 mg $100g^{-1}$ FJ) and all other treatments (Table 5.3). When averaged over different treatments mean levels of tartaric acid were significantly higher (20.57 mg $100g^{-1}$ FJ) in four weeks cold stored 'Bright Pearl' nectarine fruit than two weeks stored ones (11.47 mg $100g^{-1}$ FJ). The interaction between different treatments and cold storage period was found to be significant for the levels of tartaric acid in 'Bright Pearl' nectarine fruit (Table 5.3).

5.3.8.4. Fumaric acid

When averaged over both cold storage periods mean levels of fumaric acid were significantly higher in the 'Bright Pearl' nectarine fruit coated with chitosan (1.5%) emulsion alone (9.63 mg $100g^{-1}$ FJ), followed by the fruit coated with chitosan (1.5%) emulsion loaded with 2.0 mM SA (9.50 mg 100g-1 FJ) as compared to all other treatments (Table 5.3). When averaged over different treatments mean level of fumaric acid was significantly higher (9.54 mg $100g^{-1}$ FJ) in two weeks cold stored 'Bright Pearl' nectarine fruit than four week cold stored fruit (7.12 mg $100g^{-1}$ FJ). The interaction between different treatments and cold storage period was statistically not significant for the levels of fumaric acid in 'Bright Pearl' nectarine fruit (Table 5.3).

5.3.8.5. Succinic acid

The treatments of chitosan emulsion, SA or OA alone and chitosan emulsion loaded with SA or OA did not significantly influence mean levels of succinic acid in cold stored fruit (Table 5.3). When averaged over different treatments mean levels of succinic acid were significantly higher in the four weeks cold stored fruit (5.65 mg 100g-1 FJ) than two weeks cold stored fruit (4.12 mg $100g^{-1}$ FJ) in 'Bright Pearl'

nectarine fruit. The interaction between different coating treatments and cold storage period was also found to be non-significant for the levels of succinic acid in 'Bright Pearl' nectarine fruit (Table 5.3).

5.3.8.6. Total organic acids

When averaged over both cold storage periods mean concentration of total organic acids in 'Bright Pearl' nectarine fruit was significantly higher in the fruit coated with chitosan alone (1.5%) emulsion (1.68 g $100g^{-1}$ FJ) followed by chitosan emulsion loaded with SA (2.0 mM) (1.62 g $100g^{-1}$ FJ) compared to control (1.27 g $100g^{-1}$ FJ) and all other treatments (Table 5.3). When averaged over different treatments mean level of total organic acids was significantly higher (1.52 g $100g^{-1}$ FJ) in four weeks cold stored 'Bright Pearl' nectarine fruit than two weeks stored ones (1.44 g $100g^{-1}$ FJ). The interaction between different treatments and cold storage period was found to be significant for the levels of total organic acids in 'Bright Pearl' nectarine fruit (Table 5.3).

5.3.9. Vitamin C

When averaged over both cold storage periods, mean level of vitamin C was significantly highest (11.59 mg $100ml^{-1}$ FJ) in the 'Bright Pearl' nectarine fruit coated with chitosan (1.5%) emulsion loaded with SA (2.0 mM) compared to control (10.48 mg $100ml^{-1}$ FJ) and all other treatments (Table 5.4). Meanwhile, the fruit coated with chitosan (1.5%) emulsion loaded with OA (2.0 mM) and chitosan emulsion alone resulted in significantly lowest mean levels of vitamin C (9.53 mg $100ml^{-1}$ FJ and 9.78 mg $100ml^{-1}$ FJ respectively) compared with control and all other treatments. A significant decrease in mean concentration of vitamin C was noted from second to fourth week of cold storage periods (0.93-fold) in 'Bright Pearl' nectarine fruit. The interaction between different treatments and cold storage period was found to be significant for the levels of vitamin C in 'Bright Pearl' nectarine fruit (Table 5.4).

5.3.10. Total antioxidants

Untreated fruit showed significantly lowest levels of mean total antioxidants (40.04 µM Trolox 100 ml^{-1} FJ) when averaged over storage periods as compared to all other coating treatments (Table 5.4). 'Bright Pearl' nectarine fruit treated with OA (2.0 mM) alone exhibited significantly highest level of total antioxidants (45.00 µM

Trolox 100 ml^{-1} FJ and 46.05 µM Trolox 100 ml^{-1} FJ) following two and four weeks of cold storage periods respectively as compared with control and all other treatments (Table 5.4). When averaged over different treatments, mean levels of total antioxidants were significantly higher in the four weeks cold stored fruit (43.37 µM Trolox 100 ml^{-1} FJ) than two weeks cold stored fruit (42.61 µM Trolox 100 ml^{-1} FJ) in 'Bright Pearl' nectarine fruit. The interaction between different treatments and cold storage period was found to be significant for the levels of total antioxidants in 'Bright Pearl' nectarine fruit (Table 5.4).

5.3.11. Disease incidence

Two-week cold stored Bright Pearl nectarine fruit did not show any symptoms of diseases irrespective of the treatments, whilst symptoms of diseases were noted only on four week cold stored fruit (Fig. 5.3). The nectarine fruit coated with emulsion of chitosan (1.5%) alone exhibited lowest percentage disease incidence (2.5%) after four weeks of cold storage period, followed by chitosan emulsion loaded with 2.0 mM SA (5%) and the fruit coated with SA (2.0 mM) alone (7.5%). Untreated fruit exhibited significantly ($P \leq 0.05$) highest percentage disease incidence (22.5 %) as compared to all other treatments except 2.0mM OA (12.50%) (Fig. 5.3).

Table 5.3. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of citric acid, malic acid, tartaric acid, fumaric acid, succinic acid and total organic acids following two and four weeks cold storage period in 'Brigh Pearl' nectarine fruit.

	Citric acid (mg $100g^{-1}$ FJ)			
Treatments	2 weeks	4 weeks	Mean (T)	LSD ($P \leq 0.05$)
Control	178	171	174 bc	Treatments (T) = 0.082, Storage period (SP) = 0.047, T x SP = 0.11
Chitosan	190	198	194 a	
Chitosan + salicylic acid	182	195	188 a	
Chitosan + oxalic acid	173	184	178 b	
Salicylic acid	160	174	167 cd	
Oxalic acid	149	171	160 d	
Means (SP)	172 b	182 a		

	Malic acid (mg 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	751.4	512.2	631.8 c	Treatments (T) = 75.1, Storage period (SP) = 43.3, T x SP = 106.2
Chitosan	951.6	863.8	907.7 ab	
Chitosan + salicylic acid	838.9	1036.7	937.8 a	
Chitosan + oxalic acid	862.5	359.6	611.0 c	
Salicylic acid	911.2	811.0	861.1 ab	
Oxalic acid	756.6	920.0	838.3 b	
Means (SP)	845 a	751 b		
	Tartaric acid (mg 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	9.03	15.10	12.06 d	Treatments (T) = 2.21, Storage period (SP) = 1.27, T x SP = 3.12
Chitosan	15.40	23.93	19.66 ab	
Chitosan + salicylic acid	12.15	18.08	15.11 c	
Chitosan + oxalic acid	13.08	22.10	17.59 b	
Salicylic acid	10.98	29.25	20.11 a	
Oxalic acid	8.18	14.95	11.56 d	
Means (SP)	11.47 b	20.57 a		
	Fumaric acid (mg 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	7.50	6.25	6.88 c	Treatments (T) = 1.31, Storage period (SP) = 0.75, T x SP = NS
Chitosan	11.25	8.00	9.63 a	
Chitosan + salicylic acid	10.00	9.00	9.50 a	
Chitosan + oxalic acid	10.00	6.00	8.00 bc	
Salicylic acid	10.75	7.25	9.00 ab	
Oxalic acid	7.75	6.25	7.00 c	
Means (SP)	9.54 a	7.12 b		
	Succinic acid (mg 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	3.87	5.09	4.48 b	Treatments (T)= NS, Storage period (SP) = 0.47, T x SP = NS
Chitosan	4.51	6.62	5.57 a	
Chitosan + salicylic acid	4.14	5.46	4.80 ab	
Chitosan + oxalic acid	4.11	6.07	5.09 ab	
Salicylic acid	4.40	5.29	4.85 ab	
Oxalic acid	3.68	5.34	4.51 b	
Means (SP)	4.12 b	5.65 a		

	Total organic acids (g 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	1.327	1.208	1.27 d	Treatments (T) = 0.11, Storage period (SP) = 0.06, T x SP = 0.15
Chitosan	1.610	1.749	1.68 a	
Chitosan + salicylic acid	1.448	1.796	1.62 ab	
Chitosan + oxalic acid	1.461	1.173	1.32 d	
Salicylic acid	1.524	1.544	1.53 bc	
Oxalic acid	1.282	1.641	1.46 c	
Means (SP)	1.44 b	1.52 a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates, ten fruits per replication.

Table 5.4. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on vitamin C and total antioxidants, following two and four weeks cold storage period in 'Bright Pearl' nectarine fruit.

Vitamin C (mg 100 ml^{-1} FJ)				
	Cold storage period			
Treatment	2 weeks	4 weeks	Mean (T)	LSD ($P \leq 0.05$)
Control	10.93	10.02	10.48 d	Treatments (T) = 0.28, Storage period (SP) = 0.16, T x SP = 0.40
Chitosan	10.06	9.49	9.78 e	
Chitosan + salicylic acid	12.28	10.90	11.59 a	
Chitosan + oxalic acid	9.81	9.24	9.53 e	
Salicylic acid	11.45	11.09	11.27 b	
Oxalic acid	11.24	10.29	10.77 c	
Means (SP)	10.96 a	10.17 b		
Total antioxidant (µM Trolox 100 ml^{-1} FJ)				
Control	40.16	39.91	40.04 e	Treatments (T) = 0.44, Storage period (SP) = 0.25, T x SP = 0.62
Chitosan	41.63	42.53	42.08 d	
Chitosan + salicylic acid	43.38	43.88	43.63 b	
Chitosan + oxalic acid	41.93	43.28	42.60 c	
Salicylic acid	43.55	44.56	44.05 b	
Oxalic acid	45.00	46.05	45.53 a	
Means (SP)	42.61 b	43.37 a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates, ten fruits per replication.

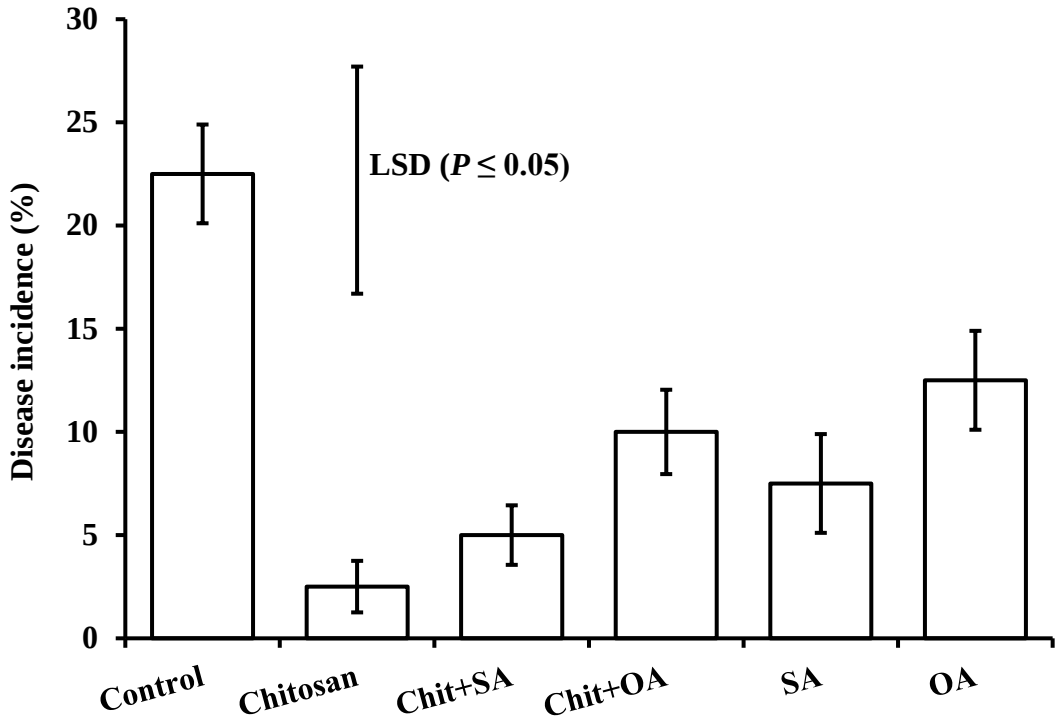


Figure 5.3. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on percentage disease incidence on the 'Bright Pearl' nectarine fruit following four weeks cold storage period. Vertical bars represent SE, n = four replicates, ten fruits per replication.

5.4. Discussion

The edible coatings have been examined in improving the appearance, reducing weight loss, extending storage life and maintaining fruit quality of the fresh horticultural produce during postharvest handling, with a variable degree of success (Baldwin et al., 1995; Petersen et al., 1999; Cha and Chinnan, 2004; Valverde et al., 2005). Some beneficial effects of chitosan coating alone on maintaining fruit quality have been reported on various fruits such as peach (Li and Yu, 2001), strawberry (Vu et al., 2011) and papaya (Asgar et al., 2011). Postharvest application of SA has shown to reduce postharvest losses of horticulture commodities on several fruits such as peach, pear, apple (Mo et al., 2008) and strawberry (Shafiee et al., 2010). Recently, OA treatment has been applied for food preservation and delays the loss of firmness, delays ripening and reduces ethylene product in mango fruit (Zheng et al., 2007b). However there is no information on the effects of postharvest application of

chitosan, SA, OA alone and in their combination on the nectarine fruit quality. Therefore, the effects of postharvest application of chitosan emulsion, SA or OA alone and chitosan emulsion loaded with SA or OA on cold storage life and quality of nectarine fruit were investigated.

5.4.1. Ethylene production

The treatment of chitosan (1.5%) emulsion loaded with SA (2.0 mM), SA (2.0 mM) alone and the treatment of chitosan (1.5%) emulsion loaded with OA (2.0 mM) significantly ($P \leq 0.05$) suppressed the climacteric ethylene production in four week cold stored nectarine fruit as compared to control and all other treatments (Fig. 5.1). Earlier, reduction in ethylene production in chitosan coated tomatoes, cucumbers and bell pepper fruits have also been reported (El Ghaouth et al., 1992b). Ethylene biosynthesis is dependent on the presence of O_2 (Abeles et al., 1992) and chitosan coating hinders the entry of oxygen into the fruit which ultimately reduces the level of endogenous ethylene (Noh, 2005). Chitosan (1.5%) emulsion loaded with SA (2.0 mM) and chitosan emulsion loaded with OA (2.0 mM) were more effective in suppressing the climacteric ethylene production in nectarine fruit compared to chitosan (1.5%) emulsion, OA and SA alone and may possibly be due to the additive effects of chitosan and OA or SA in supressing climacteric ethylene production. Earlier, postharvest application of SA has also been reported to retard ethylene biosynthesis by decreasing the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (Babalar et al., 2007). Similarly, in this study the fruit coated with SA alone and SA loaded with chitosan showed suppressed climacteric ethylene production in four week cold stored nectarine fruit as compared to control and all other treatments. Previously, Huang et al (2013) also reported that OA suppressed the ethylene production and delayed climacteric ethylene peak in banana fruit during storage and in jujube fruit (Wang et al., 2009). However, lower production rate of ethylene from sugar apple fruit by using SA treatment has been reported (Mo et al., 2008) which supports the result of this study.

5.4.2. Weight loss

Loss of fruit weight as a result of moisture loss through the skin is regulated by water pressure gradient between the fruit tissue and the storage atmosphere as well as storage temperature (Ghasemnezhad et al., 2010). Rate of metabolic activity such as respiration also contributes to weight loss during postharvest phase of the produce

(Tareen et al, 2012). The beneficial effect of SA alone (30.39%), chitosan emulsion alone (31.30%) and the combined treatment of chitosan emulsion loaded with OA (31.56%) coating in reducing the loss of nectarine fruit weight may be attributed to the influence of chitosan coatings acting as barriers to moisture loss and protecting fruit skin from mechanical injuries, sealing small wounds and thereby delaying dehydration (Ribeiro et al., 2007) (Fig. 5.2). Similarly, peach fruit treated with SA or OA alone has earlier been reported to reduce weight loss (Tareen, 2011). Prevention of the loss of weight by using chitosan coatings has also been reported in cucumber and pepper (El Ghaouth et al., 1992b), longan fruit (Jiang and Li, 2001), banana and mango (Kittur et al., 2001) and strawberries (Ribeiro et al., 2007).

5.4.3. Firmness

Higher firmness was recorded in the fruit coated with chitosan emulsion alone (46.38 N), SA alone (45.56 N) and the chitosan emulsion loaded with SA (42.60 N) compared to the control 'Bright Pearl' nectarine fruit (Table 5.1). Fruit softening in nectarine is related to the increased activities of polygalacturonase and pectin esterase involved in cell wall modification (Manganaris et al., 2005b). Possibly, the higher firmness in the coated fruit may be attributed to reduced loss of water from the cells, inhibition of conversion of water-insoluble pectin to water-soluble pectin and reduced activity of polygalacturonidase (Tareen, 2011; Weichmann, 1987). Wang et al. (2006) also reported higher flesh firmness of 'Beijing' peaches treated only with higher SA concentration.

5.4.4. SSC, TA and SSC:TA ratio

All the coating treatments including chitosan emulsion, SA and OA alone and chitosan emulsion loaded with SA or OA reduced mean SSC % in the fruit as compared to the control (Table 5.1). Lower mean SSC/TA ratio and higher mean TA were recorded in the fruit coated with chitosan emulsion and SA alone as well as chitosan emulsion loaded with SA or OA except OA alone as compared to the untreated fruit (Table 5.1). The reduction in SSC and SSC/TA ratio in coated fruit followed by cold storage as compared to the control may be attributed to the retardation of fruit ripening process due to these treatments. Similarly chitosan coating showed significant effect on the reduction of SSC and TA value in nectarine by slowing down the senescence process (Asgar et al., 2011; Chiabrando and Giacalone, 2013). Han et al. (2004) also reported that the chitosan coating slows

down the ripening and changes in the level of titratable acidity in raspberry and strawberry fruit. Decreased loss of acidity in chitosan coated peaches has also been reported by Li and Yu (2001) and Maftoonazad et al. (2008). It may also be argued that various coating treatments may have influenced SSC, TA and their ratio in nectarine fruit possibly through regulation of respiration rate, sugar and acid metabolism in the fruit.

5.4.5. Individual sugars and organic acids

As expected, sucrose, fructose and glucose were quantified from 'Bright Pearl' nectarine fruit, and sucrose was a dominant sugar followed by fructose and glucose. Similarly, sucrose, fructose and glucose as the major sugar components have been reported earlier in stone fruits along with some individual saccharides such as stachyose (Sozzi, 2004), sorbitol (Cantín et al., 2009), raffinose (Ledbetter et al., 2006), rhamnose (Kovács and Németh-Szerdahelyi, 2002), arabinose, galactose, and xylose (Gross and Sams, 1984). Concentration of total sugars significantly increased in all treatments except chitosan coated fruit and chitosan loaded with OA of 'Bright Pearl' nectarine fruit (Table 5.2) where the production of ethylene, loss of firmness and loss of weight were also considerably high and these metabolic functions might have utilized the sugar components in OA coated fruit (Gul et al., 1990). Youssef et al. (2002) reported that the concentration of reducing sugars (glucose and fructose) remains higher in coated mango fruit due to slower ripening processes which is in agreement with the observations of the current study where most of the coating treatments also showed higher concentration of reducing sugars along with lower rate of metabolic activities (Table 5.2). As sucrose is a major sugar component in nectarine fruit and its concentration increases in ripe nectarine fruit due to increased activity of sucrose-phosphate synthase (Hubbard et al., 1991), the coating treatments may have enhanced the activity of sucrose-phosphate synthase but the exact mechanism is yet to be investigated. Amongst organic acids in the 'Bright Pearl' nectarine fruit, malic acid and citric acid are dominant followed by tartaric acid, fumaric acid and succinic acid. Earlier, citric acid and malic acid are reported to be major organic acids in *Prunus* fruits (Le Dantec et al., 2010; Wu et al., 2011). Fumaric acid, tartaric acid and succinic acid have also been identified in different Prunus fruits (Flores et al., 2012). Most of the treatments resulted in increased levels of total organic acids in the 'Bright Pearl' nectarine fruit (Table 5.3). The influence

of these coatings on metabolism of sugars and organic acids is not well understood and thus warrants further investigation.

5.4.6. Vitamin C

Higher mean concentration of vitamin C (11.59 mg 100 mL^{-1} FJ) was noted in four weeks cold stored 'Bright Pearl' nectarine fruit coated with chitosan emulsion loaded with SA (Table 5.4). Ruoyi et al. (2005) reported that the combination of chitosan coating with $CaCl_2$ inhibits ascorbic acid oxidases (ASA-POD), polyphenol oxidase (PPO), peroxidase (POD) and polygalacturonase (PG) activities which contribute to maintain a relatively higher level of vitamin C in 'Zhonghuashoutao' peach fruit. Similarly, higher levels of vitamin C in mango fruit coated with chitosan have been attributed to slow ripening rate of the coated fruit (Abbasi et al., 2009). Edible coatings reduce the permeability of O_2 and CO_2 (Srinivasa et al., 2002). Peach fruit treated by SA or OA alone showed higher level of vitamin C in the treated fruit (Tareen, 2011) which is similar to the findings of the current study of OA treated 'Bright Pearl' nectarine.

5.4.7. Total antioxidants

Increased total phenolics and antioxidant activity have been reported in chitosan (0.5%) coated apricot fruit during cold storage (Ghasemnezhad et al., 2010). Significant effect of SA and OA alone have also been noted in improving the anti-oxidative capacity of peach fruit (Zhang et al., 2007a; Tareen, 2011; Khademi and Ershadi, 2013), papaya (Setha et al., 2000), mandarin (El-hilali et al., 2003), sugar apple fruit (Mo et al., 2008) and grapes (Asghari et al., 2013). These observations are in agreement with the experimental results obtained from the current study, where higher average level of total antioxidants have been noted in the OA treated 'Bright Pearl' nectarine fruit (45.51 µM Trolox 100 ml^{-1} FJ) compared to the control and all other treatments (Table 5.4). Ripe 'Bright Pearl' fruit which were treated with SA alone, OA alone and chitosan emulsion loaded with SA and OA showed significantly higher level of total antioxidants (Table 5.4) compared to the uncoated fruit. Similarly, postharvest dip application of OA and SA has also been reported to improve total antioxidant activity in 'Elberta' peach fruit compared to untreated fruit (Khademi and Ershadi, 2013). The exact mechanism by which chitosan, SA and OA influence levels of total antioxidants in nectarine fruit during cold storage is not known and warrants investigation.

5.4.8. Disease incidence

Four weeks cold stored 'Bright Pearl' nectarine fruit which were coated with chitosan emulsion (1.5%), the chitosan emulsion loaded with SA and SA 2.0 mM alone or the chitosan emulsion loaded with OA exhibited significantly lower percentage disease incidence (2.5, 5, 7.5, 10 % respectively) compared to the control (22.5%) and the treatment of OA alone (12.5%) (Fig. 5.3). Chitosan application possibly may have inhibited the germination of fungal spores, mycelium growth on the fruit surface and /or may have activated the defence response of the fruit tissue by activating pathogen-related (PR) gene function, such as chitinases, chitosanase, β-glucanases, lignin and callose as reported earlier by Zhang et al., (2011). Previous studies have indicated that chitosan could effectively inhibit postharvest diseases on various horticultural commodities (Romanazzi et al., 2003; Bautista-Banos et al., 2006; Zhang et al., 2011; Bal, 2013). The beneficial effects in reducing percentage disease incidence in fruit treated with SA may possibly be attributed to enhanced resistance of the nectarine fruit to various pathogens as reported earlier by Asghari and Aghdam (2010). Similarly, Khademi and Ershdi, (2013) and Asghari and Aghdam, (2010) also reported that SA treatment lowered fruit decay in plum fruit. Moreover, SA has been reported to decrease decay in peaches, pears, apples, nectarines and bananas by retarding fruit softening processes and starch degradation (Mo et al., 2008). Chitosan alone was a most effective treatment in reducing percentage disease incidence in 'Bright Pearl' nectarine fruit as compared to the application of chitosan emulsion loaded with SA.

5.5. Conclusion

Coating of chitosan loaded with SA was proved to be more effective in reducing ethylene production, and maintaining higher levels of fructose, malic acid and vitamin C. The application of chitosan, SA or OA alone was more effective in maintaining various fruit quality parameters such as reducing loss of weight, firmness and disease incidence compared to the chitosan loaded with SA or OA . In conclusion, the 'Bright Pearl' nectarine fruit coated with chitosan emulsion or SA or OA alone were more effective in maintaining quality of four weeks cold stored fruit compared to chitosan emulsion loaded with SA or OA and the poroposed hypothesis of this thesis that chitosan emulsion loaded with SA or OA is more effective than chitosan emulsion, SA and OA alone was refuted. In the future, tests could be

undertaken to determine the effect of nano-emulsions of chitosan alone or loaded with SA or OA to regulate ethylene production and maintain fruit quality in nectarine fruit.

CHAPTER 6

Postharvest quality of Japanese plums (*Prunus salicina* Lindl. cv 'Angeleno' and 'Tegan Blue') fruit at ambient temperature influenced by coating of chitosan emulsion, salicylic acid or oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid

Summary

Plum is a climacteric fruit and has a short storage life. The present study was conducted to investigate the effects of chitosan emulsion, salicylic acid (SA) or oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on ethylene production, fruit ripening and quality of plum cv. Angelino and 'Tegan Blue' under ambient conditions. The cultivars 'Angelino' and 'Tegan Blue' plum fruit showed genotypic differences in response to the coating material used in the current study. The chitosan emulsion (1.5%) coating alone significantly ($P \leq 0.05$) suppressed mean ethylene production (0.046 and 0.69 µmol Kg^{-1} h^{-1}) compared to the control (0.054 and 1.12 µmol Kg^{-1} h^{-1}) and other treatments in cv. Angelino and 'Tegan Blue' respectively. The fruit coated with chitosan (1.5%) exhibited significantly ($P \leq 0.05$) higher fruit firmness (37.29 N), sucrose (1.51g 100 ml^{-1} FJ), total sugars (11.06 g 100 ml^{-1} FJ), level of fumaric acid (2.03 mg 100 ml^{-1} FJ), malic acid (2.50 g 100 ml^{-1} FJ) and total antioxidants (45.74 13 µM Trolox 100 ml^{-1} FJ) in ripe 'Angelino' plum fruit. Higher level of tartaric acid (3.55 mg 100 ml^{-1} FJ), vitamin C (35.78 mg 100 ml^{-1} FJ), soluble solids concentration (SSC) (18.35%) and SSC: titratable acidity (TA) ratio in ripe 'Angelino' fruit were recorded when fruit were coated with chitosan emulsion loaded with SA. Similarly, the fruit coated with chitosan (1.5%) resulted in significantly ($P \leq 0.05$) higher fruit firmness (20.69N) in ripe 'Tegan Blue' plum fruit. Whilst, reduced weight loss (5.52%) compared to control (12.49%), increased level of sucrose (6.09 g 100 ml^{-1} FJ), total sugars (12.57 g 100 ml^{-1} FJ), malic acid (3.08 g 100 ml^{-1} FJ), succinic acid (0.59 g 100 ml^{-1} FJ), total organic acids (3.74 mg 100 ml^{-1} FJ) and vitamin C (29.94 mg 100 ml^{-1} FJ) in ripe 'Tegan Blue' fruit were recorded in the fruit coated with chitosan emulsion loaded with SA. In conclusion, chitosan emulsion coating suppressed ethylene production during ripening in both cultivars. Chitosan emulsion loaded with SA was more

effective in maintaining most parameters of fruit quality in 'Tegan Blue' whilst chitosan emulsion alone seemed to be more effective in cv 'Angelino'.

6.1. Introduction

Plum fruit has a short postharvest life and cold storage at 0°C is recommended to extend its storage life and maintain quality (Crisosto et al., 2004). Commercially, in the supply chain the plum fruits are kept at (0-5 °C and 80-95% relative humidity). These storage conditions retard loss of fruit firmness; minimise weight loss and incidence of postharvest diseases; however these conditions are coupled with development of chilling injury (CI) symptoms in some cultivars (Crisosto et al., 1999). CI symptoms in plum fruit expressed as flesh browning, mealiness, flesh translucency, red pigment accumulation also known as bleeding, and loss of flavour are genotype as well as storage temperature dependent (Crisosto et al., 2004). The CI symptoms in plum fruit reduce consumer acceptance (Crisosto et al., 2004).

Various methods to extend postharvest life and minimize postharvest losses in fruit crops have been tested. In many cases growers rely on alternative methods such as physical, controlled atmosphere, and biological control which reduce pesticide usage (Eshel et al., 2009). However, optimum storage temperatures higher than 7.5 °C have been used during the supply chain of plum fruit, depending upon cultivar, to minimise the development of CI and its adverse effects on fruit quality (Crisosto and Garner, 2008). Controlled atmosphere (CA) storage at 7.5 °C has been tested to minimise CI but reduced concentrations of oxygen (3 –5 kPa), higher concentrations of CO_2 (10–15 kPa) and extended storage periods also lead to fruit softening and development of 'off' flavours (Crisosto and Garner, 2008).

Conventionally, various fungicides are used in controlling postharvest diseases. Pathogens develop resistance to fungicides when used over a long time and are also reported to be harmful to human health (Stefano et al., 2009; Ren and Shaoying, 2013). The use of natural compounds which enhance resistance of the host and/or with fungistatic action, low residue and environmentally friendly offers attractive alternatives to the application of fungicides. Therefore, new alternatives for controlling postharvest diseases which have good efficacy, low residues and little or no toxicity to non-target organisms are in urgent demand. Different kinds of edible coating materials (alginate, cellulose, chitosan, chitin, lipids, mucilage, milk protein, starch, wax, and zein) are used and act as barriers to loss of moisture and diffusion of

oxygen during the postharvest phase of the fruit (Falguera et al., 2011). Beneficial effects of edible coatings, SA and OA in improving the attractiveness of the produce, extending shelf life as well as maintaining fruit quality have been described in previous chapter 4. The effects of postharvest coating of chitosan loaded with SA or OA on plum fruit ripening now warrants investigation. It was hypothesised that chitosan emulsion loaded with SA or OA will be more effective in retarding plum fruit ripening at ambient temperature compared to their application alone. Therefore, this study is aimed at investigating the influence of chitosan coating, SA and OA alone or chitosan emulsion loaded with SA or OA on rate of ethylene production and change in various biochemical fruit quality parameters in 'Angelino' and 'Tegan Blue' Japanese plum fruit.

6.2. Materials and methods

6.2.1. Plant material

Plums (*Prunus salicina* Lindl. cv 'Angelino' and 'Tegan Blue') fruit were picked at commercial maturity (SSC = 15.77% and 16.27%, fruit firmness = 54.71 N and 56.39 N, ethylene production = 0.92 and 0.09 µmol kg^{-1} h^{-1} respectively) from Balingup (33° 47' S/ 115° 59' E) Western Australia. Following the harvest, fruit of medium size, no visible symptoms of diseases and physiological disorders were transported to the Horticulture Research Laboratory, Curtin University, Perth, WA.

6.2.2. Treatments and experimental design

In the first experiment, the 'Angelino' plum fruit were coated by spraying aqueous emulsion containing chitosan (1.5%), solution of SA (2.0 mM) or OA (2.0 mM) alone or the chitosan emulsion (1.5%) loaded with SA (2.0 mM) or OA (2.0 mM) and Tween 20 (0.25%) as a surfactant on the fruit surface. Uncoated fruit were kept as a control. Following the treatments, the fruit were kept at ambient temperature (20 ± 1 °C) and relative humidity (60 ± 5%). The experiments followed completely randomized design (CRD) with four replications and 10 fruits in each replication. Ethylene production was determined daily for nine days. Fruit weight loss, firmness, soluble solids concentration (SSC), titratable acidity (TA), ratio of SSC and TA, total and individual sugars and organic acids, vitamin C, total antioxidants and disease incidence of the fruit were determined two weeks after treatments. The second experiment was conducted on 'Tegan Blue' plum fruit. All the experimental

conditions, treatments, and design and observations recorded were similar to the first experiment.

6.2.3. Determination of production of ethylene

Ethylene production was determined by following the method described earlier by Pranamornkith et al. (2012) and detailed in Chapter 3, Section 3.4. The rate of ethylene was determined by using an ETD 300 ethylene detector (Sensor sense B.V, Nijmegen, The Netherlands). Each fruit sample was weighed before transferring into the cuvettes [1.0 L air-tight jar, fitted with a rubber septum (SubaSeal, Sigma-Aldrich Co., St. Louis, USA)] and all the cuvettes were kept tight to prevent leakage. Before connecting flow to the cuvette, it was ensured that the outlet of the cuvette was not blocked. Each sample ran for 20 min with an air flow rate of 4 L hr^{-1}. The determined ethylene was expressed as µmol kg^{-1} h^{-1}. The detailed method has been described earlier in Chapter 3, Section 3.4.

6.2.4. Determination of loss of fruit weight

The loss of fruit weight was calculated as the percentage of fresh fruit weight against initial weight at harvest as described in Chapter 3, Section 3.4

6.2.5. Determination of fruit firmness

The firmness of fruit pulp was estimated by employing a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK) fitted with a horizontal square base table (15 cm × 15 cm) and by following the previously detailed method in Chapter 3, Section 3.7. A small slice (~2 mm thick) of fruit skin was removed and the firmness were recorded on the opposite sides of the equatorial region of individual fruit by puncturing a 7/16 inch Magness-Taylor probe, with a 500 N load cell. The crosshead speed, depth, trigger and compression were maintained at 100 mm min^{-1}, 7.5 mm, 1 N and 75%, respectively. The firmness of the fruit was calculated as newtons (N).

6.2.6. Determination of SSC, TA and SSC: TA ratio

The juice was extracted from ten randomly selected fruit using a fruit juicer (Model JE8500, Sunbeam Corp. Ltd., Botany, Australia) to determine the SSC and TA. SSC: TA ratio was calculated by dividing SSC by the corresponding TA value. Details of the procedures have been described in Chapter 3, Section 3.8.

6.2.7. Determination of individual sugars and organic acids

Individual sugars and organic acids from the fruit juice were determined by using an HPLC system (Waters 1525, Milford Corp., MA, USA). The detailed method has previously been described in Chapter 3, Section 3.9.

6.2.8. Determination of vitamin C

Vitamin C concentrations in the juice were estimated using the method described earlier by Malik and Singh (2005). Vitamin C concentration was calculated as mg vitamin C 100 ml^{-1} fruit juice. The detailed method has also been explained in Chapter 3, Section 3.10.

6.2.9. Determination of total antioxidants

Total antioxidants in the fruit juice were estimated by employing the modified method described earlier by Brand-William et al. (1995) and Pham (2009). The levels of total antioxidants were expressed as µM trolox equivalent antioxidant activity (TEAC) 100 ml^{-1} FJ basis. Details of the procedures have been described in Chapter 3, Section 3.11.

6.2.10. Determination of disease incidence

The rate of disease incidence was expressed as a percentage and determined by examining the fruit regularly and regarded as infected if visible symptoms were observed. More details have previously been described in Chapter 3, Section 3.13.

6.2.11. Statistical analysis

The data on ethylene production during fruit ripening and other parameters determined two-weeks after treatments were subjected to two-way and one-way analysis of variance (ANOVA) respectively, using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted experimental station, UK). The effects of different coating treatments, ripening period and their interactions for ethylene production were evaluated within ANOVA. The effects of different coating treatments on fruit quality parameters were also assessed. The detailed are included in Chapter 3, Section 3.14.

6.3. Results

6.3.1. Ethylene production

'Angelino' plum fruits coated with chitosan emulsion (1.5%) alone or chitosan emulsion loaded with 2.0 mM SA (2.0 mM) delayed climacteric ethylene peaks (0.046 and 0.062 µmol Kg^{-1} h^{-1} respectively) until the sixth day after application of treatments compared to the ethylene peaks of SA (2.0 mM), OA (2.0 mM) and chitosan loaded with OA (0.050, 0.046 and 0.052 µmol Kg^{-1} h^{-1} respectively) treatments which appeared on 5, 4 and 3 days respectively (Fig.6.1A). Meanwhile, the control fruit exhibited climacteric ethylene peak (0.054 µmol Kg-1 h^{-1}) on the second day after treatment. All the treatments except OA (2.0 mM) did not affect the time of appearance of climacteric ethylene peak during ripening period in 'Tegan Blue' plum fruits (Fig. 6.1B). However, the 'Tegan Blue' plum fruits coated with chitosan emulsion alone exhibited suppressed climacteric ethylene peak (0.69 µmol Kg^{-1} h^{-1}) as compared to the control (1.12 µmol Kg^{-1} h^{-1}) and all other treatments (Fig.6.1B). When averaged over ripening period mean ethylene production was significantly suppressed by all the treatments compared to untreated 'Angelino' plum fruit (Fig. 6.2A). The treatments of chitosan emulsion (1.5%) alone and OA (2.0 mM) alone significantly ($P \leq 0.05$) suppressed mean ethylene production (0.041, 0.041 µmol Kg^{-1} h^{-1} respectively) compared to the control (0.051 µmol Kg^{-1} h^{-1}) and other treatments in 'Angelino' plums (Fig. 6.2A). 'Tegan Blue' plum fruit coated with chitosan alone exhibited significantly lowest mean ethylene production (0.32 µmol Kg^{-1} h^{-1}) followed by the fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM SA (0.45 µmol Kg^{-1} h^{-1}) as compared to the control (0.64 µmol Kg^{-1} h^{-1}) and all other treatments (Fig. 6.2B).

6.3.2. Weight loss

All the treatments have significantly ($P \leq 0.05$) reduced weight loss as compared to the untreated 'Angelino' fruit. In 'Tegan Blue', the weight loss was significantly highest in control fruit as compared to all other treatments except the fruit coated with chitosan loaded with OA (2.0 mM) and OA alone. The fruit coated with chitosan loaded with SA (2.0 mM) in both 'Angelino' and 'Tegan Blue' plum cultivars exhibited significantly ($P \leq 0.05$) lowest weight loss (13.89% and 5.52% respectively) compared to the control fruit (27.21% and 12.49% respectively) and all other treatments (Fig. 6.3A and B).

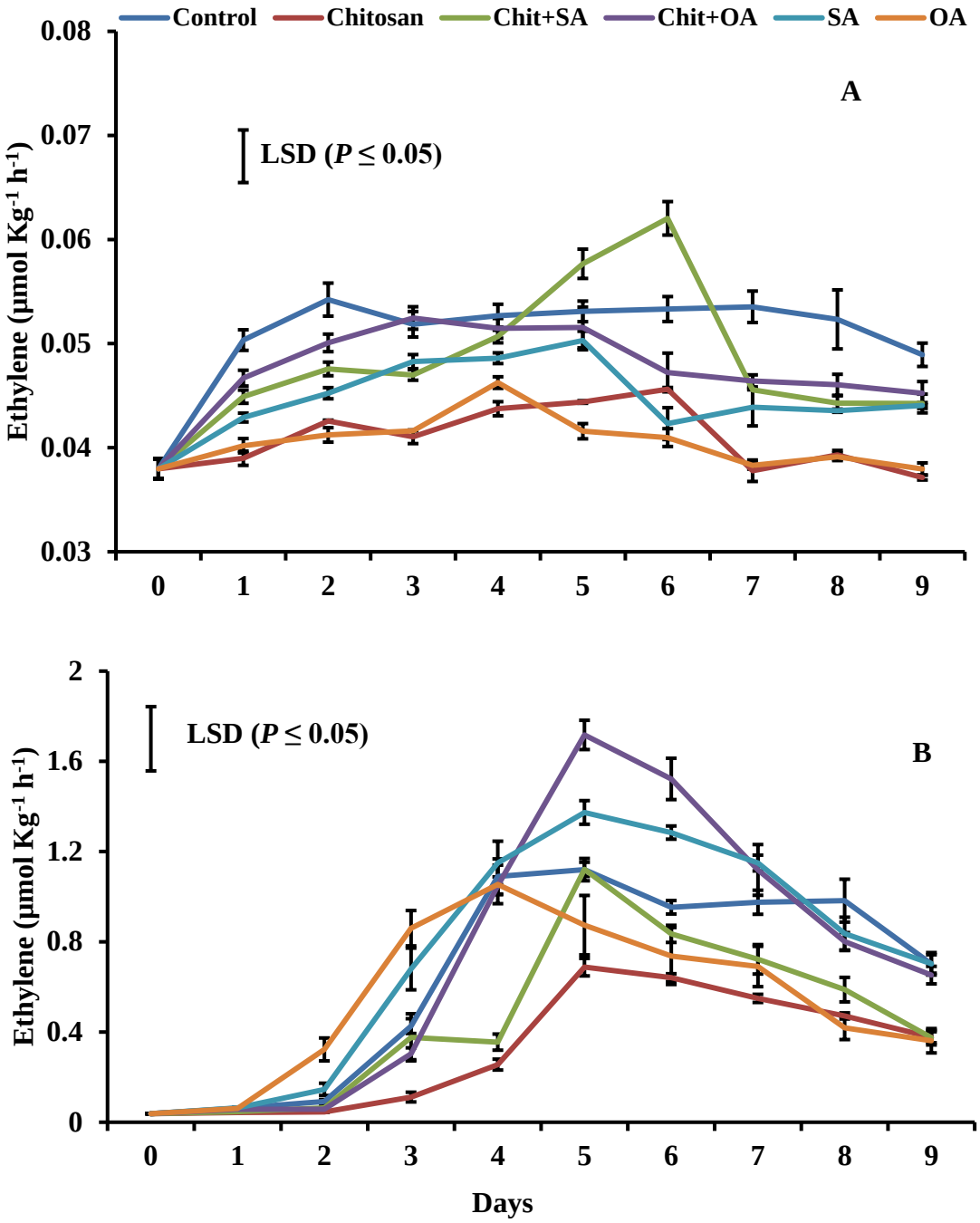


Figure 6.1. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on ethylene production during fruit ripening period in (A) 'Angelino' and (B) 'Tegan Blue' cultivars of plum. Vertical bars represent SE, n = four replicates, two fruits per replication.

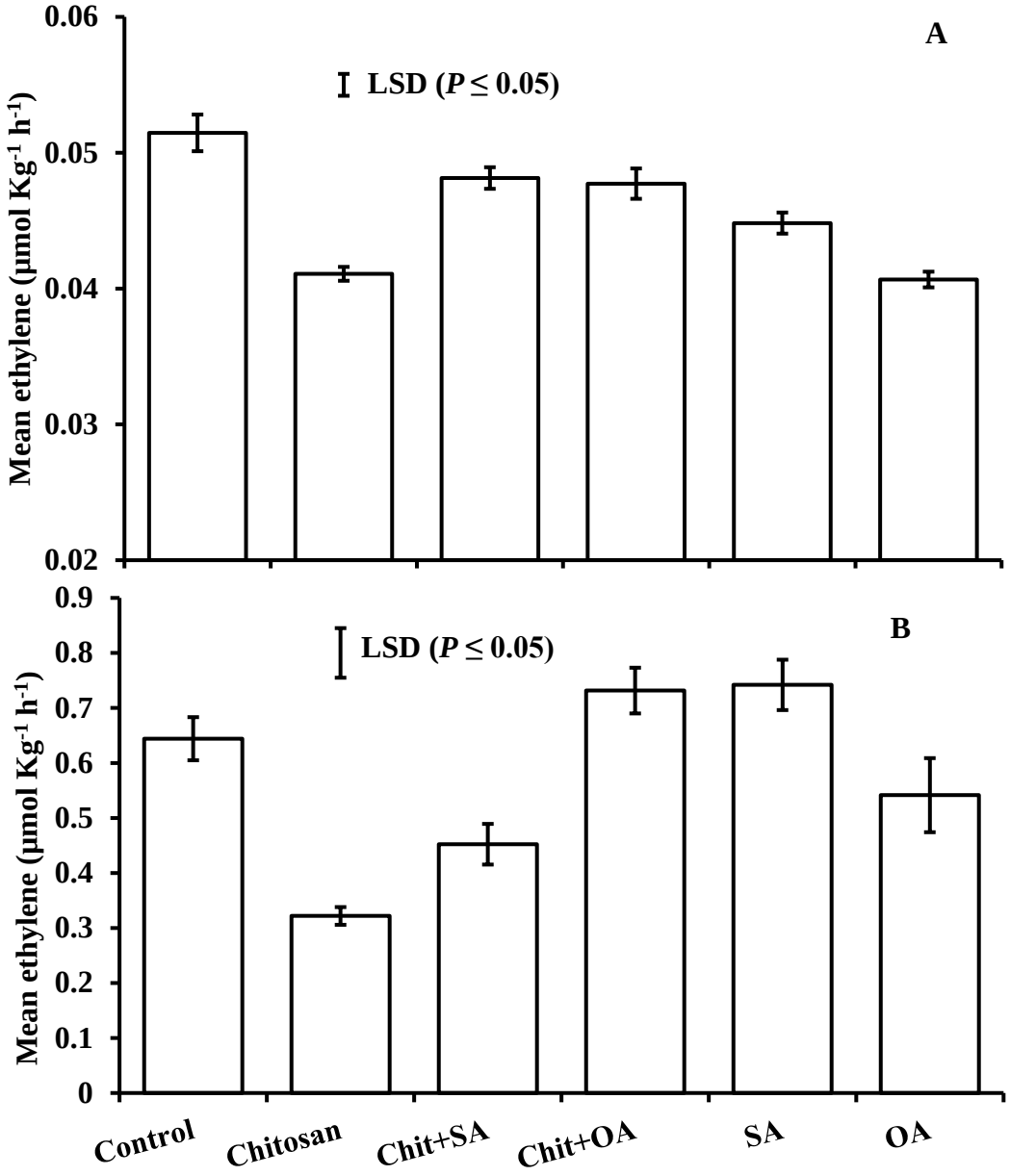


Figure 6.2. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on mean ethylene production during fruit ripening period in (A) 'Angelino' and (B) 'Tegan Blue' cultivars of plum. Vertical bars represent SE, n = four replicates, two fruits per replication.

6.3.3. Firmness

All the treatments reduced loss of fruit firmness two weeks after treatments in 'Angelino' and 'Tegan Blue' cultivars of plums as compared to the control (29.27 and 10.78 N respectively). The fruit firmness was significantly higher in both 'Angelino' and 'Tegan Blue' cultivars of plum when fruit coated with chitosan

(1.5%) emulsion alone (37.29 and 20.69 N respectively) and chitosan loaded with SA (2.0 mM) (35.29 and 18.06 N respectively) as compared to control and all other treatments (Fig. 6.4A and B).

6.3.4. Soluble solids concentration (SSC)

'Angelino' plum fruits coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) exhibited significantly higher SSC (18.35%) compared to the control (17.02%) and all other treatments (Fig. 6.5A). Whilst, all the coating treatments except 2.0 mM OA significantly reduced SSC in 'Tegan Blue' plum compared to the control fruit (19.1%) (Fig.6.5B)

6.3.5. Titratable acidity (TA)

All the treatments did not significantly affect the levels of titratable acidity (TA) in 'Angelino' plum fruit but TA was highest (1.06%) in the plum fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM SA (Fig. 6.6A). However, lowest level of titratable acidity (0.99%) was recorded in 'Angelino' plum fruit which were coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) compared to the control and all other treatments (Fig 6.6A). All of the treatments significantly affected levels of TA in the 'Tegan Blue' plum fruit. The fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM SA exhibited significantly highest levels of TA (1.65%) compared to the control fruit (1.26%) and all other treatments (Fig. 6.6 B). A lower level of TA (1.23%) was recorded in the 'Tegan Blue' plum fruit coated with OA (2.0 mM) alone compared to the control and all other treatments (Fig. 6.6B).

6.3.6. SSC: TA ratio

All the treatments did not significantly influence the SSC:TA ratio in the 'Angelino' plum fruit (Fig. 6.7A). Meanwhile in cv. Tegan Blue', all of the treatments except OA (2.0 mM) significantly reduced SSC:TA ratio in the fruit as compared to all other treatments and control (Fig. 6.7B). However, 'Tegan Blue' plum fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) resulted in the significantly lowest level of SSC/TA ratio (10.42) compared to control (15.17) and all other treatments (Fig. 6.7B).

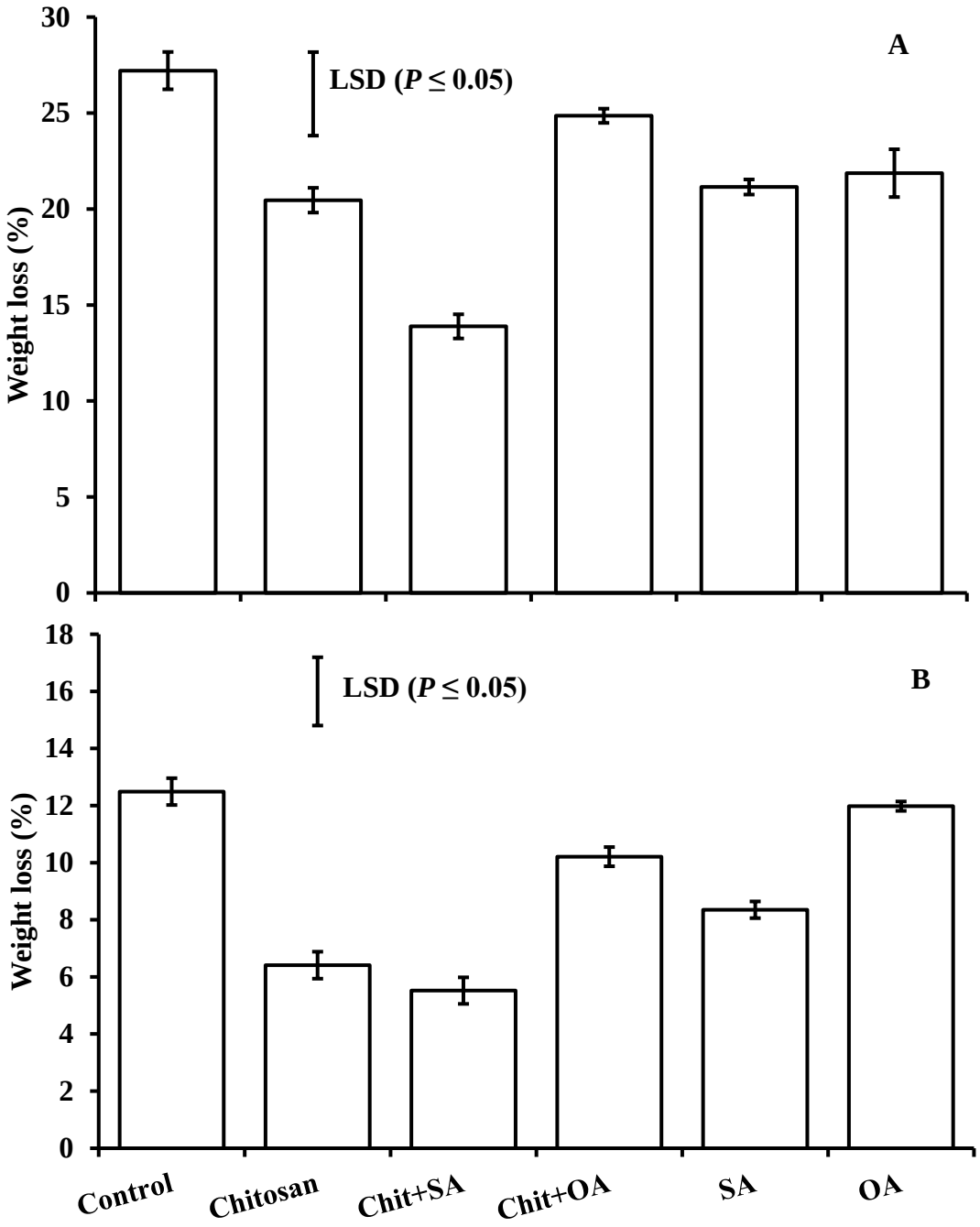


Figure 6.3. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on fruit weight loss during ripening period in (A) 'Angelino' and (B) 'Tegan Blue' cultivars of plum two weeks after treatments at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

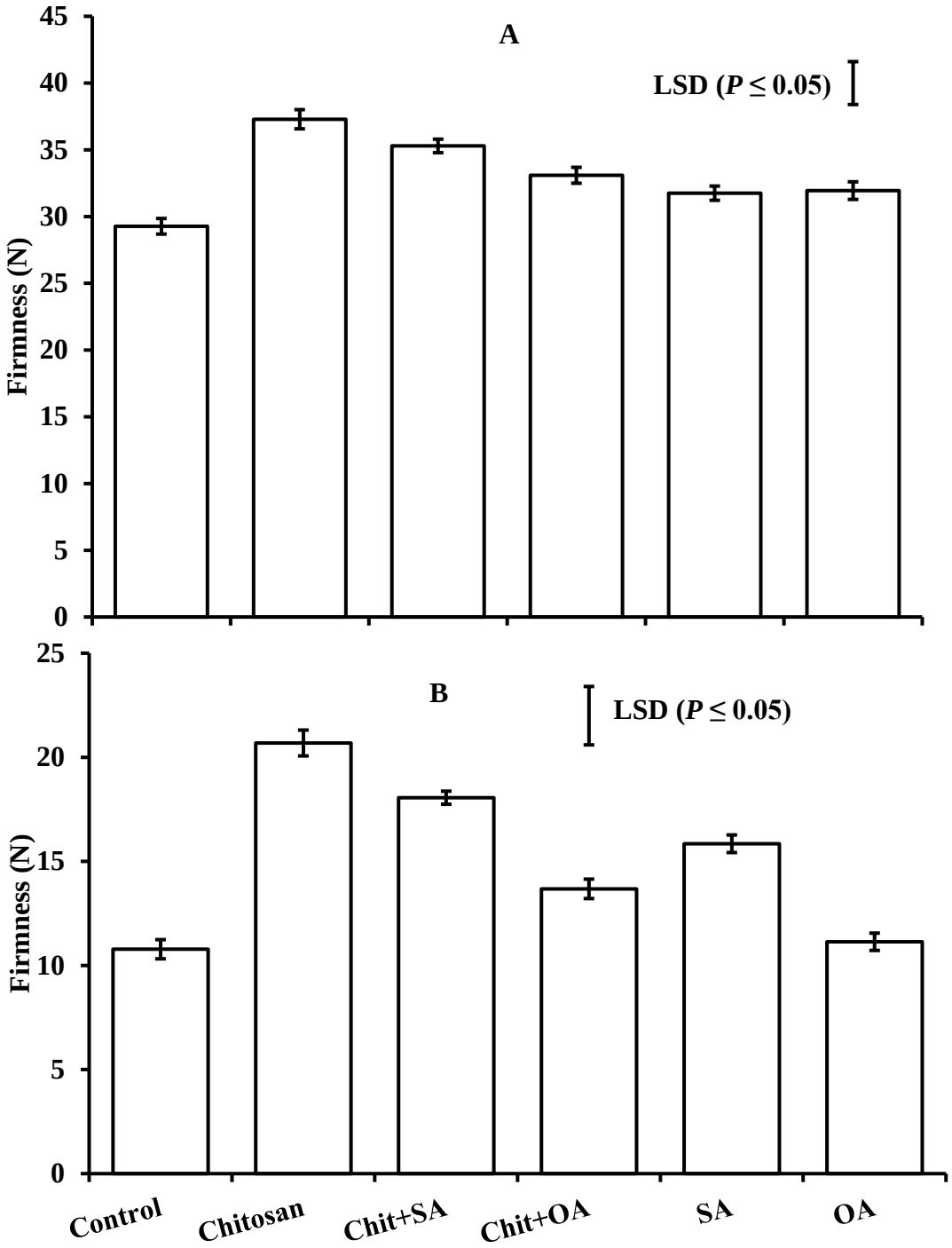


Figure 6.4. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and the chitosan emulsion loaded with SA or OA on firmness during fruit ripening period in (A) 'Angelino' and (B) 'Tegan Blue' cultivars of plum two weeks after treatments at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

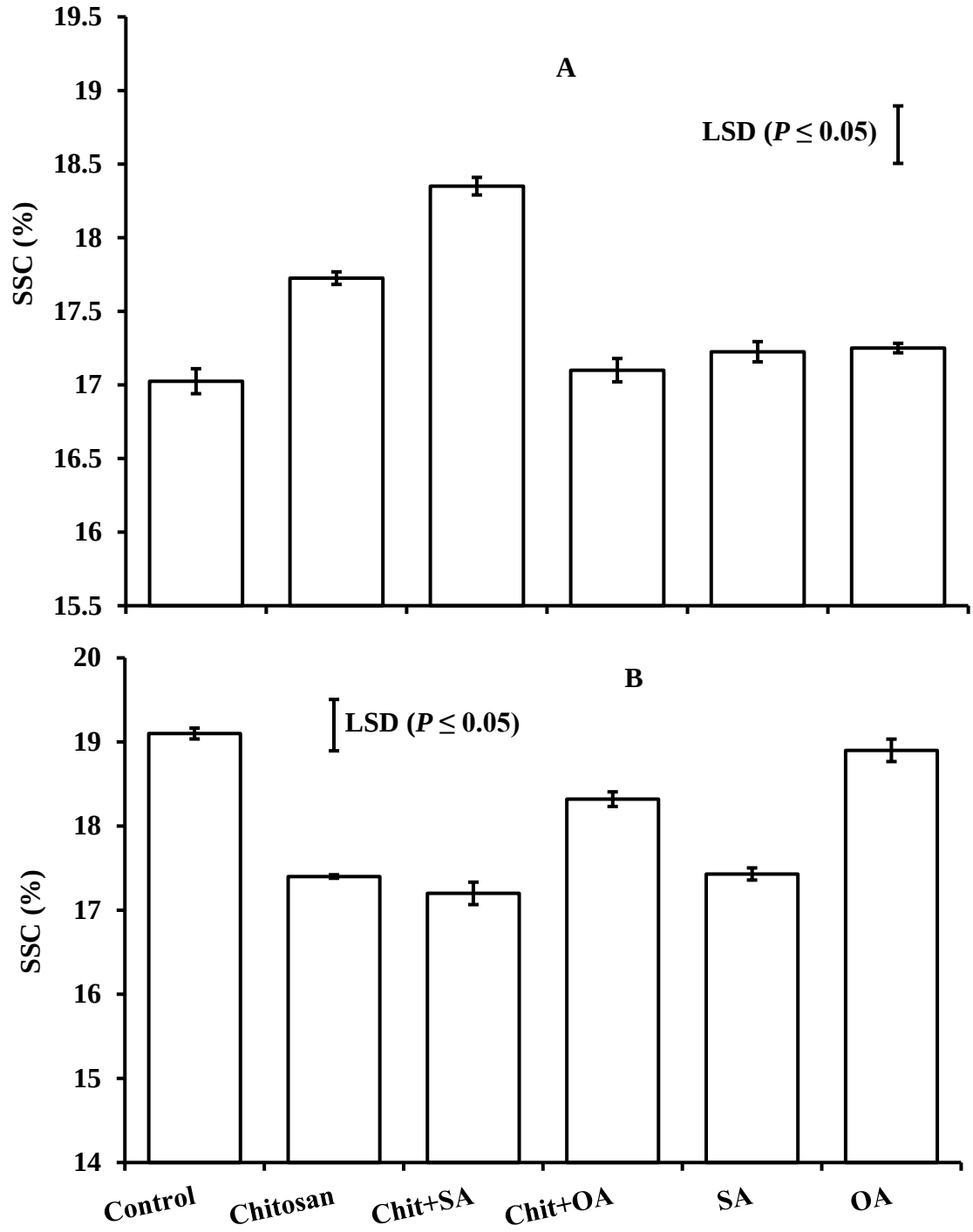


Figure 6.5. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and the chitosan emulsion loaded with SA or OA on SSC during fruit ripening period in (A) 'Angelino' and (B) 'Tegan Blue' cultivars of plum two weeks after treatments at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

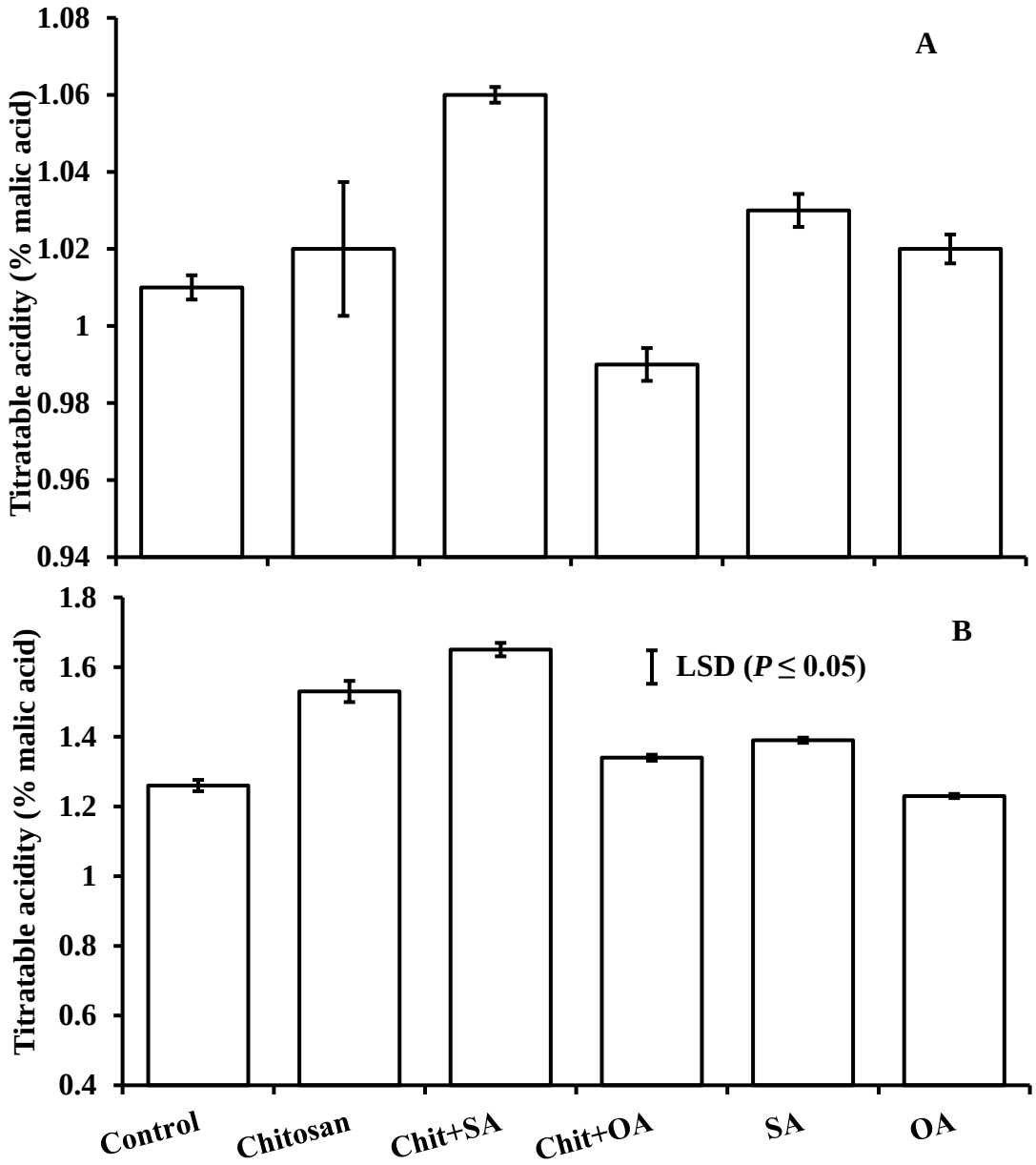


Figure 6.6. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on TA during fruit ripening period in (A) 'Angelino' and (B) 'Tegan Blue' cultivars of plum two weeks after treatments at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

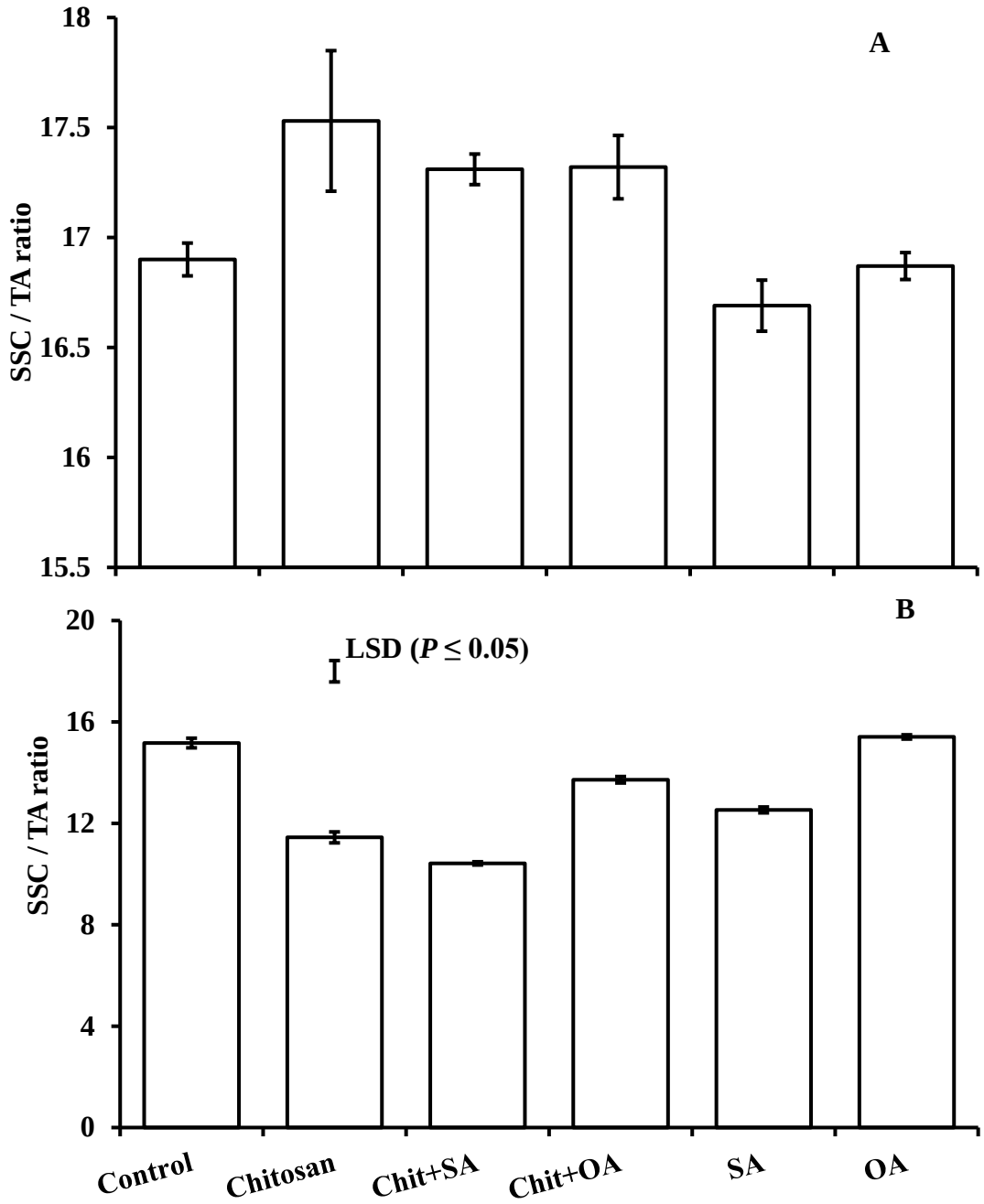


Figure 6.7. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and the chitosan emulsion loaded with SA or OA on SSA: TA ratio during fruit ripening period in (A) 'Angelino' and (B) 'Tegan Blue' cultivars of plum two weeks after treatments at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

6.3.7. Individual sugars

Fructose was found to be the major sugar component in 'Angelino' plum fruit followed by glucose then sucrose (Fig. 6.8). Whilst, in 'Tegan Blue' plum fruit, fructose was the major sugar component followed by sucrose then glucose (Fig. 6.9).

6.3.7.1. Fructose

All the treatments significantly affected levels of fructose in both 'Angelino' and 'Tegan Blue' plum fruit two weeks after the treatments. 'Angelino' plum fruit coated with chitosan alone resulted in significantly higher levels of fructose (5.21 g $100ml^{-1}$ FJ) as compared to control (4.03 g $100ml^{-1}$ FJ) and all other treatments except when the fruit were coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) (Fig. 6.8A). All the treatments reduced the levels of fructose in 'Tegan Blue' plum fruit as compared to control (5.23 g $100ml^{-1}$ FJ). However, lowest level of fructose (3.62 g $100ml^{-1}$ FJ) was observed in the fruit which were coated with chitosan emulsion (1.5%) alone compare to the control and other treatments in 'Tegan Blue' plum fruit (Fig. 6.9A).

6.3.7.2. Glucose

All the coating treatments significantly affected levels of glucose in the 'Angelino' fruit but not in 'Tegan Blue' plum fruits. 'Angelino' plum fruit coated with chitosan emulsion (1.5%) alone resulted in significantly higher levels of glucose (4.34 g $100ml^{-1}$ FJ) as compared to the control fruit (3.30 g $100ml^{-1}$ FJ) and all other treatments except chitosan emulsion (1.5%) loaded with 2.0 mM OA (Fig. 6.8B). The levels of glucose in 'Tegan Blue' plum fruit were not significantly affected by any of the treatments tested (Fig. 6.9B).

6.3.7.3. Sucrose

All the treatments showed significantly ($P \leq 0.05$) higher concentrations of sucrose in 'Angelino' plum fruit as compared to the control (Fig. 6.8C). Similarly, the 'Tegan Blue' plum fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) resulted in significantly highest levels of sucrose (6.09 g $100ml^{-1}$ FJ) compared to control (2.35 g $100ml^{-1}$ FJ) and all other treatments except the fruit coated with chitosan emulsion (1.5%) alone (Fig. 6.9C). However, lowest sucrose level (1.99 g $100ml^{-1}$ FJ) was observed in plum fruit cv. Tegan Blue treated with OA (2.0 mM) alone as compared to the control and all other treatments.

6.3.7.4. Total sugars

The treatments have significantly ($P \leq 0.05$) affected the levels of total sugars in the 'Angelino' plum fruit only and not in 'Tegan Blue'. 'Angelino' plum fruit which were coated with chitosan emulsion (1.5%) alone resulted in significantly highest levels of total sugars (11.06 g $100ml^{-1}$ FJ) as compared with control (8.29 g $100ml^{-1}$ FJ) and all other treatments except the fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM OA (Fig. 6.8D). No treatments significantly affected the levels of total sugars in the 'Tegan Blue' plum fruit (Fig. 6.9D).

6.3.8. Organic acids

Various organic acids were determined in plum fruit such as citric acid, malic acid, fumaric acid, tartaric acid and succinic acid by using HPLC. Malic acid is a major organic acid in the fruit of both 'Angelino' and 'Tegan Blue' plum cultivars (Fig. 6.10C and Fig. 6.11C).

6.3.8.1. Citric acid

The levels of citric acid in 'Angelino' plum fruit were not significantly affected by any of the treatments tested. Whilst, all the treatments tested significantly affected the levels of citric acid in 'Tegan Blue' plum fruits. The 'Tegan Blue' plum fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) and SA (2.0 mM) alone resulted in significantly higher levels of citric acid (59.87 and 59.62 mg $100ml^{-1}$ FJ respectively) compared to control (54.4 mg $100ml^{-1}$ FJ) and other treatments (Fig 6.11A).

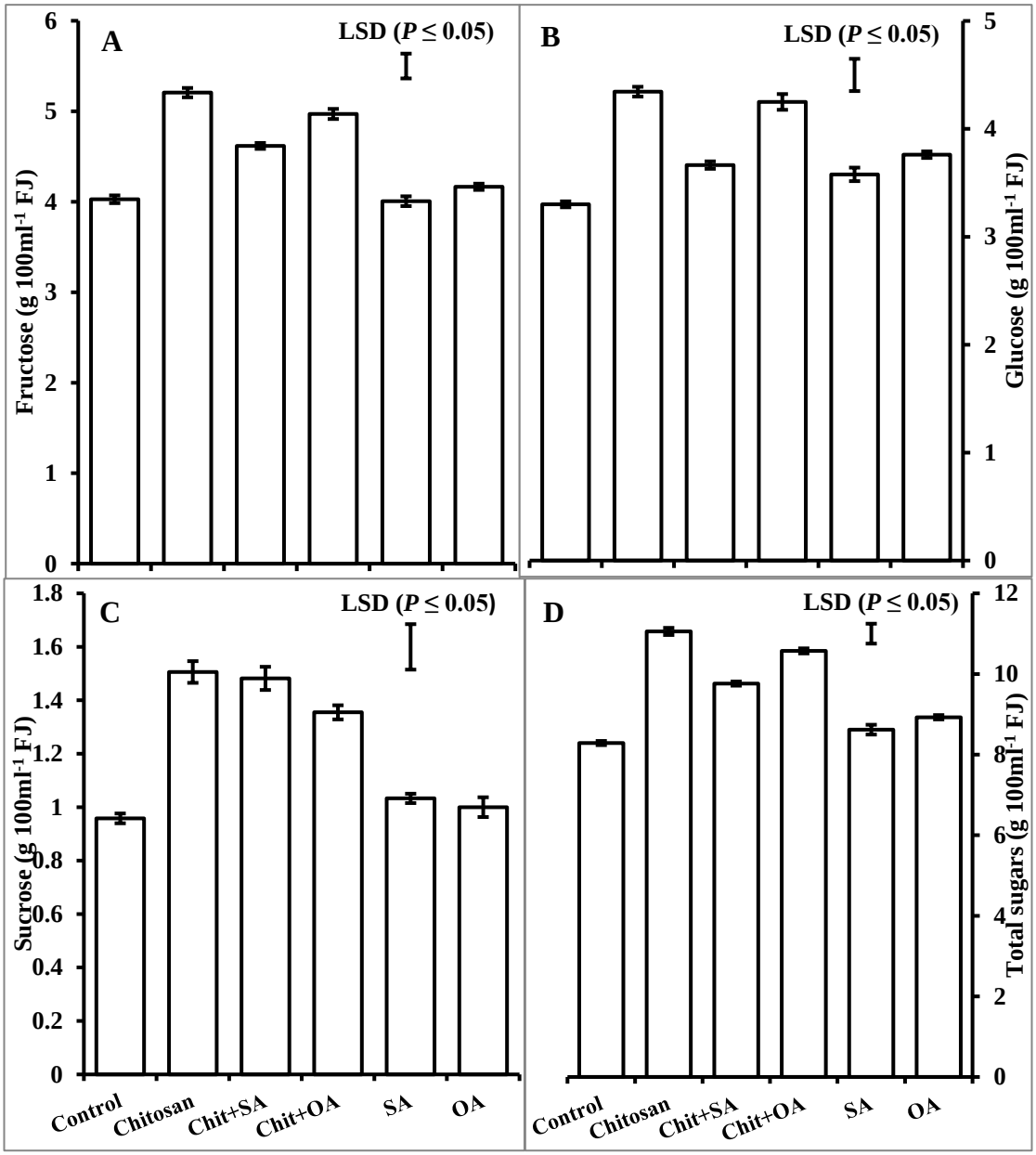


Figure 6.8. A-D. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of (A) fructose, (B) glucose, (C) sucrose and (D) total sugars in the juice of 'Angelino' plum fruit two weeks after treatments at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

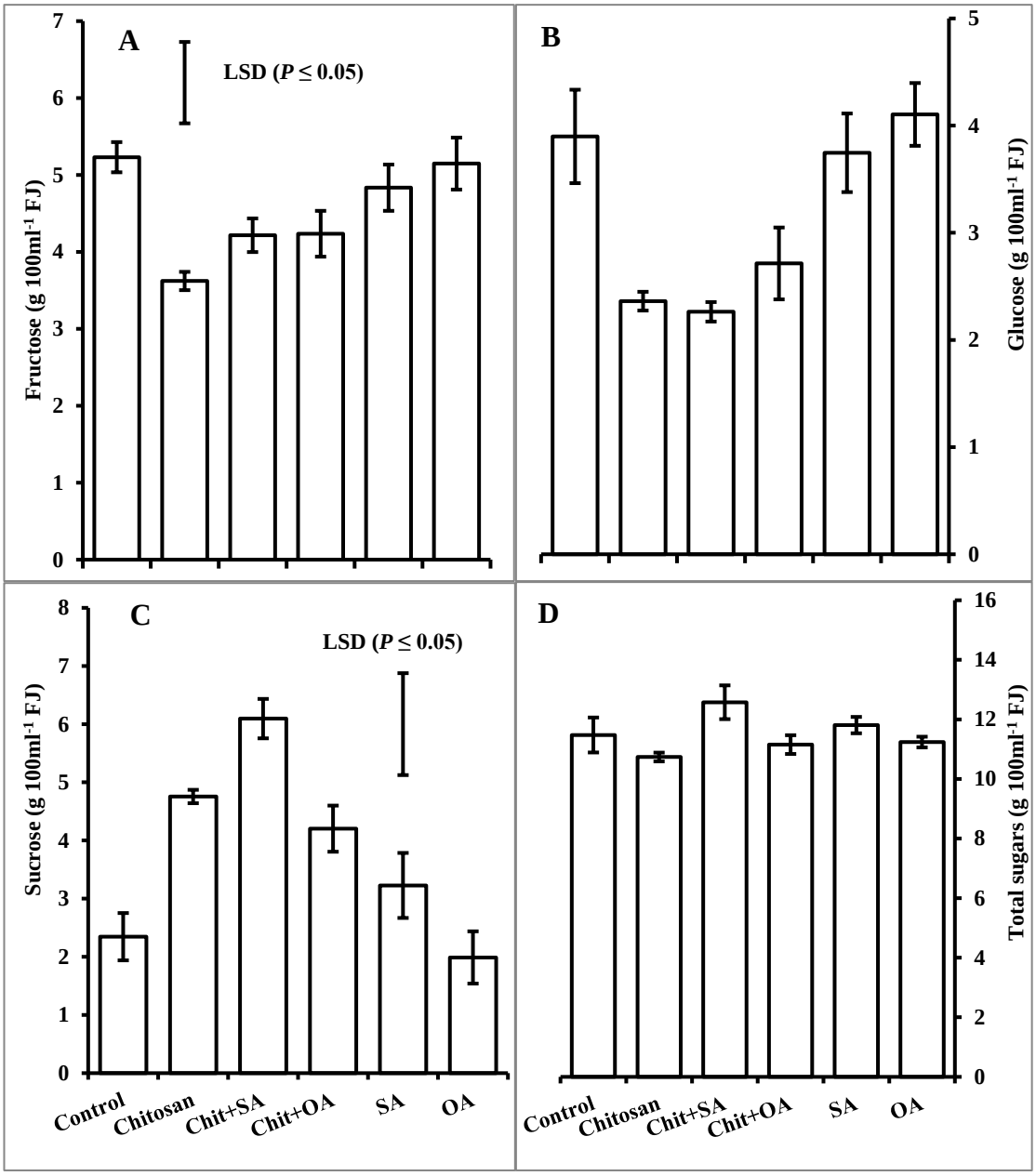


Figure 6.9. A-D. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of (A) fructose, (B) glucose, (C) sucrose and (D) total sugars in the juice of 'Tegan Blue' plum fruit two weeks after treatments at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

6.3.8.2. Fumaric acid

The level of fumaric acid in 'Angelino' plum was significantly higher (2.03 and 2.01 mg 100ml^{-1} FJ) when coated with chitosan emulsion (1.5%) alone and the chitosan loaded with SA (2.0 mM) respectively as compared to control and all other treatments (Fig. 6.10B). However, the lowest level of fumaric acid (1.92 mg 100ml^{-1}

FJ) was observed in uncoated fruit followed by 2.0 mM SA alone (1.94 mg $100ml^{-1}$ FJ) and 2.0 mM OA alone (1.96 mg $100ml^{-1}$ FJ) compared to all other treatments (Fig. 6.10B). Meanwhile, higher levels of fumaric acid (2.42 and 2.35 mg $100ml^{-1}$ FJ) were observed in the fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) and chitosan emulsion (1.5%) alone respectively in 'Tegan Blue' plum fruit compared to the control (2.00 mg $100ml^{-1}$ FJ) and all other treatments (Fig. 6.11B).

6.3.8.3. Malic acid

The level of malic acid was higher (2.50 g $100ml^{-1}$ FJ) in the ripe 'Angelino' plum fruit which were coated with chitosan emulsion (1.5%) alone as compared to control and all other treatments (Fig. 6.10C). Whilst, lowest malic acid level (2.11 g $100ml^{-1}$ FJ) was observed in the untreated fruit followed by chitosan emulsion (1.5%) loaded with SA (2.0 mM) (2.22 g $100ml^{-1}$ FJ) as compared to all other treatments in cv. Angelino. The highest level of malic acid (3.08 g $100ml^{-1}$ FJ) was noted in 'Tegan Blue' plum fruit when coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) compared to all other treatments and control (2.79 g $100ml^{-1}$ FJ) (Fig. 6.11C).

6.3.8.4. Succinic acid

The fruit coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) showed highest levels of succinic acid (0.49 g $100ml^{-1}$ FJ) in 'Angelino' plum fruit as compared to control (0.45 g $100ml^{-1}$ FJ) and all other treatments (Fig. 6.10D). However, a significantly higher level of succinic acid (0.59 g $100ml^{-1}$ FJ) was observed in cv. Tegan Blue in fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) as compared to control (0.53 g $100ml^{-1}$ FJ) and all other treatments (Fig. 6.11D).

6.3.8.5. Tartaric acid

The levels of tartaric acid in 'Angelino' plum fruit were not significantly influenced by the tested treatments. The level of tartaric acid was higher (3.55 and 3.55 mg $100ml^{-1}$ FJ) in the fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM SA and OA alone, respectively, as compared to control (3.52 mg $100ml^{-1}$ FJ) and all other treatments in 'Angelino' plum fruit (Fig. 6.10E). In 'Tegan Blue' plum fruit, a higher level of tartaric acid (1.83 and 1.78 mg $100ml^{-1}$ FJ) was noted in the control

fruit and those fruit coated with 2.0 mM SA alone, respectively, as compared to all other treatments (Fig. 6.11E).

6.3.8.6. Total organic acids

The levels of total organic acids in the juice were significantly ($P \leq 0.05$) higher (3.06 and 3.02 g 100ml^{-1} FJ) in the ripe 'Angelino' plum fruit which were coated with chitosan emulsion (1.5%) alone and chitosan emulsion (1.5%) loaded with OA respectively as compared with control and all other treatments, except when the fruit were coated with 2.0 mM SA and 2.0 mM OA (Fig. 6.10F). Meanwhile, lowest levels of total organic acids (2.63 g 100ml^{-1} FJ) were observed in control 'Angelino' plum fruit. In 'Tegan Blue', the fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM SA resulted in significantly highest level of total organic acids (3.74 g 100ml^{-1} FJ) compared with control (3.37 g 100ml^{-1} FJ) and all other treatments (Fig. 6.11F).

6.3.9. Vitamin C

Fruit coated with SA (2.0 mM) alone and chitosan emulsion (1.5%) loaded with SA showed significantly ($P \leq 0.05$) highest levels of vitamin C (36.30 mg 100 ml^{-1} FJ and 35.78 mg 100 ml^{-1} FJ respectively) in 'Angelino' plum fruit as compared to the control (33.3 mg 100 ml^{-1} FJ) and all other treatments (Fig 6.12A). The lowest level of vitamin C (30.73 mg 100 ml^{-1} FJ) was observed in the fruit coated with chitosan emulsion (1.5%) alone as compared to control and all other treatments in 'Angelino' plum fruit. In cv. Tegan Blue significantly higher concentrations of vitamin C (29.94 and 27.09 mg 100 ml^{-1} FJ) were noted when fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) and SA (2.0 mM) alone respectively, compared to control (24.51 mg 100 ml^{-1} FJ) and all other treatments (Fig. 6.12B). Whilst, the lowest level of vitamin C (23.81 mg 100 ml^{-1} FJ) in 'Tegan Blue' fruit was observed when coated with chitosan emulsion (1.5%) alone as compared to control and all other treatments.

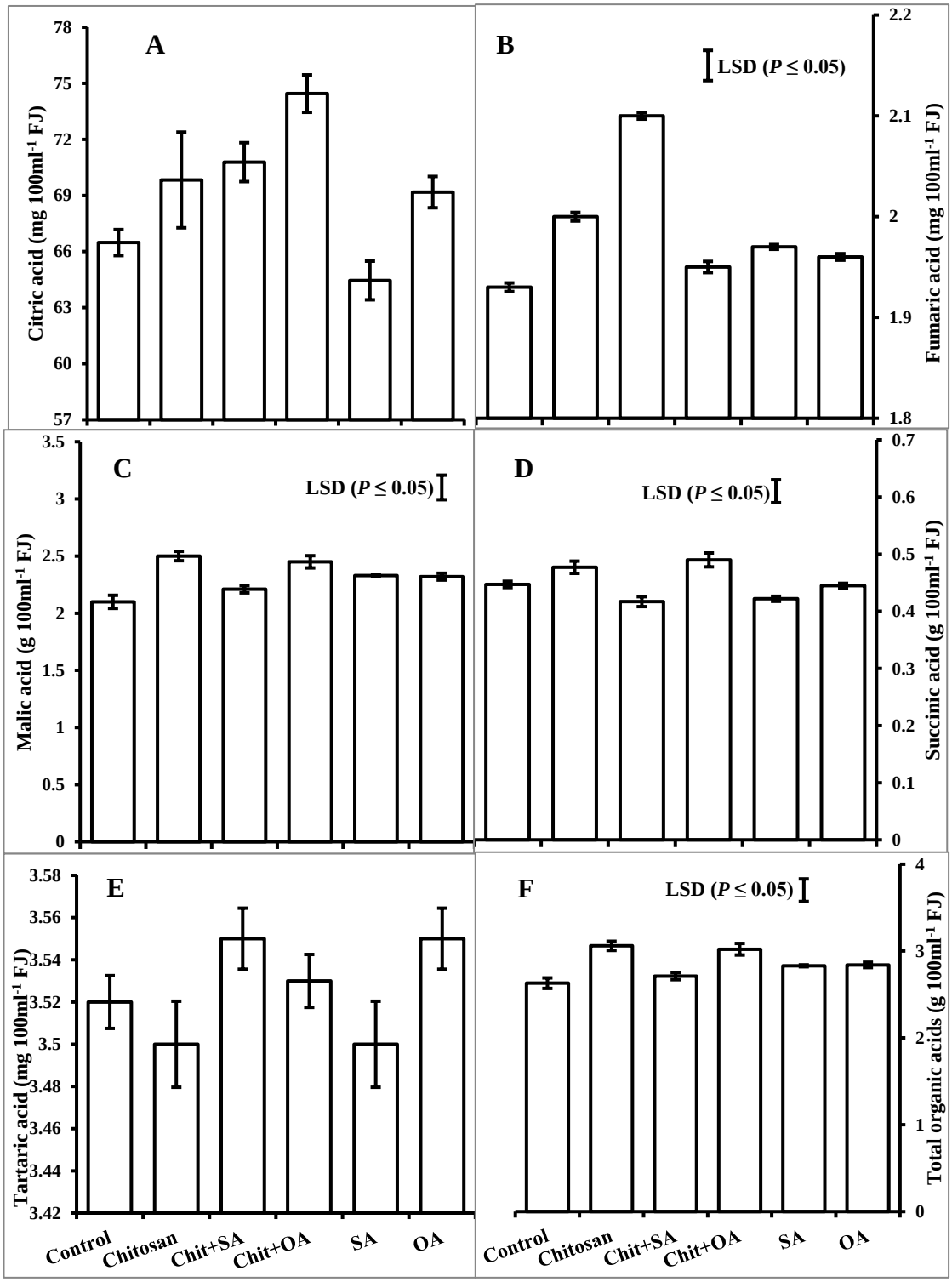


Figure 6.10. A-F. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of (A) citric acid, (B) fumaric acid, (C) malic acid, (D) succinic acid, (E) tartaric acid and (F) total organic acids in the juice of 'Angelino' plum fruit two weeks after treatments at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

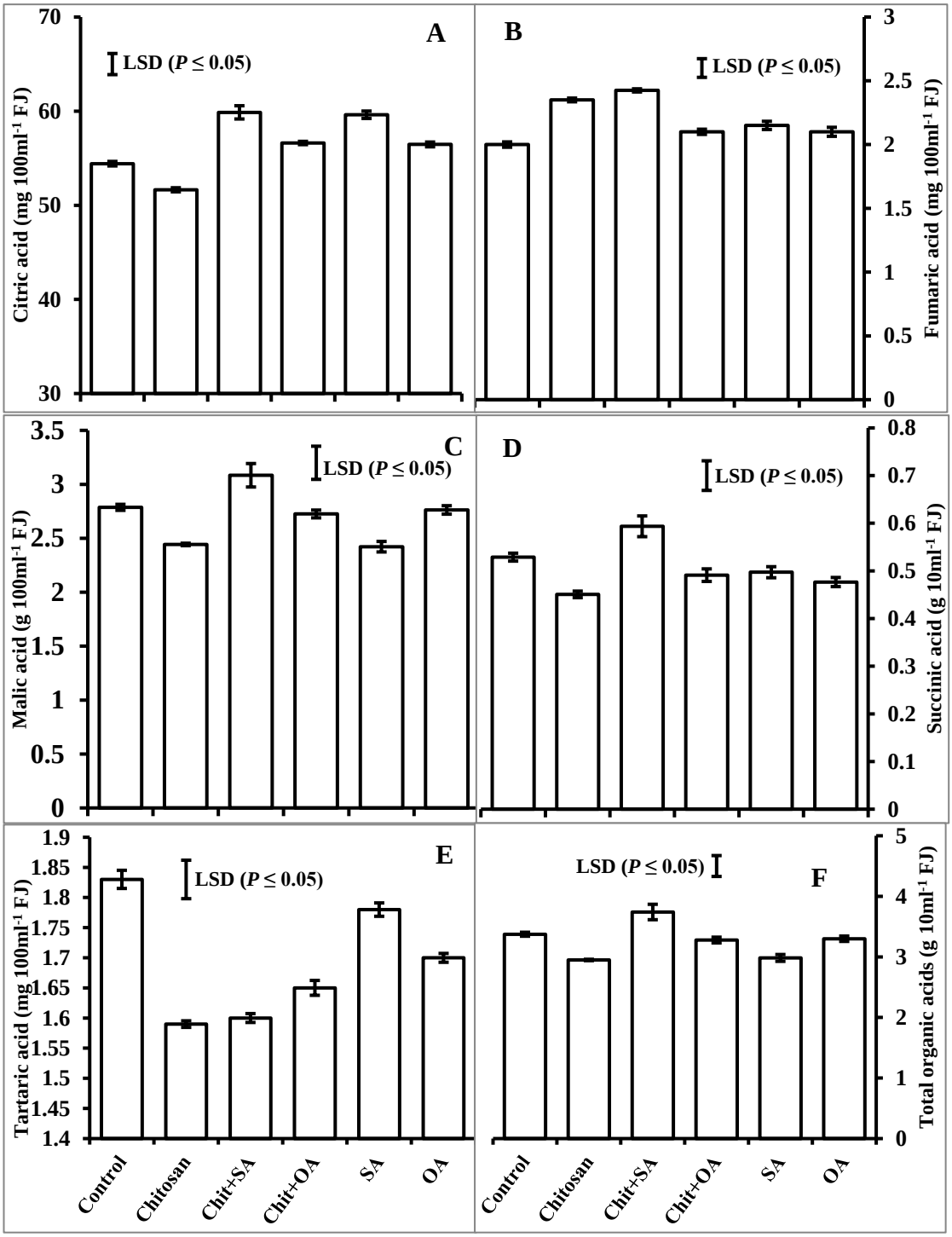


Figure 6.11. A-F. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of (A) citric acid, (B) fumaric acid, (C) malic acid, (D) succinic acid, (E) tartaric acid and (F) total organic acids in the juice of 'Tegan Blue' plum fruit two weeks after treatments at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

6.3.10. Total antioxidants

'Angelino' plum fruit coated with chitosan emulsion (1.5%) alone and chitosan emulsion (1.5%) loaded with OA (2.0 mM) showed significantly ($P \leq 0.05$) highest levels of total antioxidants (45.74 and 45.12 µM Trolox 100 ml^{-1} FJ, respectively) as compared to control (41.46 µM Trolox 100 ml^{-1} FJ) and all other treatments (Fig. 6.13A). Similarly, significantly ($P \leq 0.05$) higher levels of total antioxidants (46.26 and 44.92 µM Trolox 100 ml^{-1} FJ) were observed in 'Tegan Blue' plum fruit when coated with OA (2.0 mM) alone and chitosan emulsion (1.5%) loaded with OA (2.0 mM) respectively, as compared to control (44.07 µM Trolox 100 ml^{-1} FJ) and all other treatments (Fig. 6.13B). However, the lowest level of total antioxidants (41.96 µM Trolox 100 ml^{-1} FJ) was observed in 'Tegan Blue' plum fruit when coated with SA (2.0 mM) alone compared to control and all other treatments.

6.3.11. Disease incidence

All the coating treatments had reduced percentage disease incidence in both 'Angelino' and 'Tegan Blue' plum fruit compared to untreated fruit which exhibited significantly ($P \leq 0.05$) highest percentage disease incidence (15.25% and 20% respectively) (Fig. 6.14A and B). However, in 'Angelino', the lowest incidence of disease (4.5%) was recorded when fruit were coated with chitosan emulsion (1.5%) alone as compared to control and all other treatments (Fig. 6.14A). The 'Tegan Blue' coated with chitosan emulsion loaded with 2.0 mM SA exhibited lowest disease incidence (7.50%) as compared to control (20.0%) and all other treatments (Fig 6.14B).

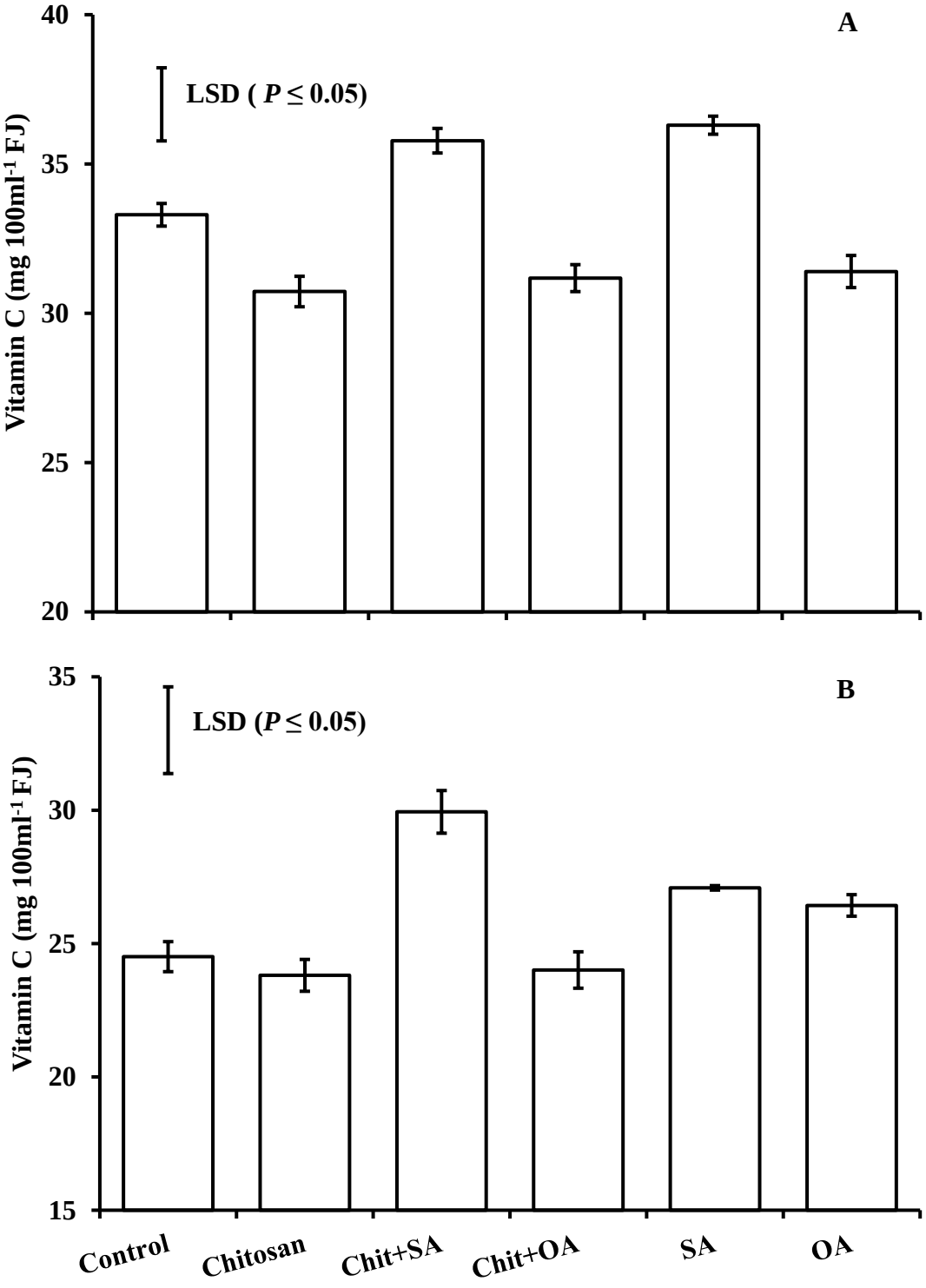


Figure 6.12. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of vitamin C two weeks after treatments in (A) 'Angelino' and (B) 'Tegan Blue' plum fruit at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

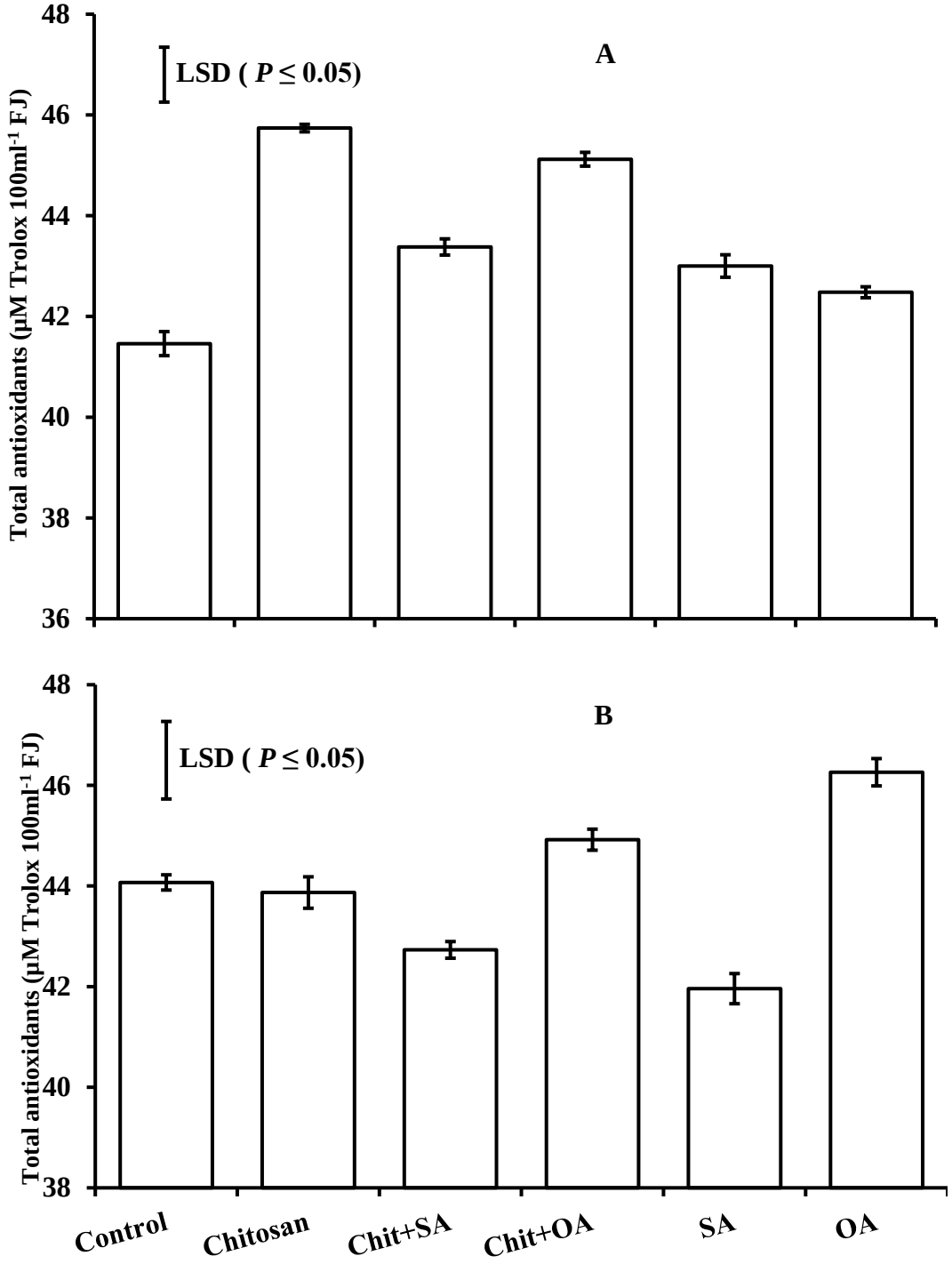


Figure 6.13. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of total antioxidants two weeks after treatments in (A) 'Angelino' and (B) 'Tegan Blue' plum fruit at ambient temperature. Vertical bars represent SE, n = four replicates, ten fruits per replication.

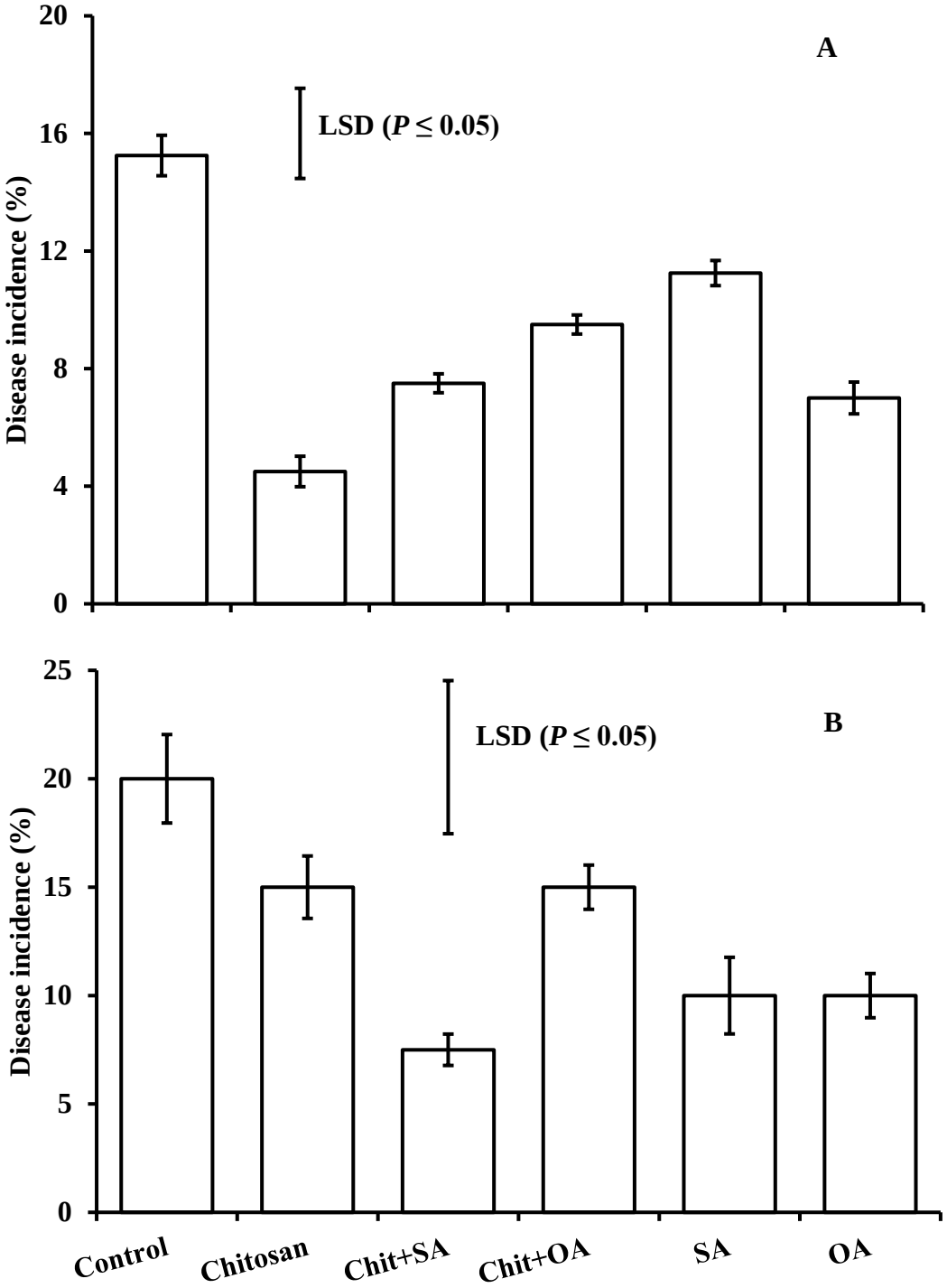


Figure 6.14. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on percentage disease incidence at ambient temperature two weeks after treatments in (A) 'Angelino' and (B) 'Tegan Blue' plum fruit. Vertical bars represent SE, n = four replicates, ten fruits per replication.

6.4. Discussion

In this experiment the effects of postharvest application of chitosan emulsion, SA, OA alone and chitosan emulsion loaded with SA or OA on ethylene production, in modulating fruit ripening and quality of plum cultivar 'Angelino' and 'Tegan Blue' at ambient conditions were investigated.

6.4.1. Ethylene production

Chitosan emulsion (1.5%) coating and OA (2.0 mM) alone significantly ($P \leq 0.05$) suppressed and delayed climacteric ethylene production compared to the control and other treatments in 'Angelino' plum fruit during ripening period (Fig. 6.1A and 6.2 A). 'Tegan Blue' plum fruit coated with chitosan alone, chitosan emulsion (1.5%) loaded with 2.0 mM SA followed by OA alone suppressed climacteric ethylene production (Fig. 6.1B and 6.2 B). Possibly, the reduction in ethylene production in chitosan coated plum fruits may be ascribed to the hindrance of the entry of oxygen into the plum and other fruits (Noh, 2005) as ethylene biosynthesis is dependent on the presence of O_2 (Abeles et al., 1992). It may also be argued that chitosan coating suppressed endogenous ethylene production by retarding the activities of key ethylene biosynthesis enzymes such as ACC oxidase and ACO synthase (Noh, 2005). Similarly, chitosan coating has also been reported to suppress ethylene production previously in different fruits such as tomatoes, cucumbers and bell peppers (El Ghaouth et al., 1992b). The suppression of ethylene production in plum fruit treated with OA alone may be tentatively possible due to the reduced activity of ethylene biosynthesis ACS and ACO enzymes, however this was not investigated in the present study. Similarly, Wu et al. (2011) also reported the reduction in ethylene production in 'Damili' plum fruit treated with 5 mM OA. The reduction in ethylene production in the plum fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM salicylic acid or SA alone may be ascribed to the reduced activity of ACC as previously reported by Zhang et al. (2003) and ACO (Leslie and Romani, 1998).

6.4.2. Weight loss

Fruit weight loss through the fruit skin is mainly associated with respiration and moisture evaporation. However, the thin skin of plum fruits makes them susceptible to rapid water loss, resulting in shriveling and rapid deterioration of quality. In the present study, the chitosan coating beneficially reduced the loss of weight in cv. Angelino and 'Tegan Blue' of plums. The fruit coated with chitosan loaded with SA

exhibited the significantly ($P \leq 0.05$) least weight loss (13.89% and 5.52%) compared to the control (27.21% and 12.49%) and other treatments, respectively, in both cultivars of plums 'Angelino' and 'Tegan Blue' (Fig. 6.3A and B). Earlier, Ribeiro et al., (2007) reported that edible coatings act as barriers thereby restricting water loss from the fruit surface. Apart from plum fruit, chitosan coatings have been effective at reducing water loss from other fruit such as litchi (Donglin et al., 1997; Dong et al., 2004), tomatoes (El Ghaouth et al., 1992b), longan fruit (Jiang and Li, 2001), banana and mango (Kittur et al., 2001), strawberries (Ribeiro et al., 2007), and plum (Bal, 2013). Similarly, the least weight loss was recorded in chitosan emulsion coated fruit in both 'Honey Fire' and 'Bright Pearl' nectarine compared to the control as reported in Chapter 4.

6.4.3. Firmness

Plum fruit suffers a rapid loss of firmness during ripening which contributes greatly to its short postharvest life. The fruit firmness was significantly ($P \leq 0.05$) higher in both 'Angelino' and 'Tegan Blue' cultivars of plum when fruit were coated with chitosan emulsion alone and loaded with SA as compared to control and all other treatments which may be associated with the reduced ethylene production (Fig. 6.4A and B). Ethylene plays an important role in fruit softening by regulating the activities of softening enzymes (PE, EGase, exo-PG and endo-PG) as previously reported in plum by Khan and Singh. (2007b). The beneficial effect of chitosan on loss of fruit firmness has also been previously reported in different fruits such as peach, Japanese pear, kiwifruit (Du et al., 1997) and citrus 'Murcott' tangor (Chien et al., 2007). Similarly, mango and pears have also been reported to be firmer when coated with chitosan (Zhu et al., 2008). However, the plum fruit firmness seems to be a genotype dependent attribute which has been noted in the current study from higher level of firmness in ripe 'Angelino' (37.29 N) than the 'Tegan Blue'(20.69 N) plum fruit treated with the chitosan emulsion (Fig. 6.4A and B). Suppressed-climacteric and climacteric type of ethylene production during fruit ripening in 'Angelino' and 'Tegan Blue' plum respectively may also have influenced fruit softening. Ehtylene plays key role in plum fruit softerning (Khan and Singh, 2007a).

6.4.4. SSC, TA and SSC: TA ratio

SSC, TA and SSC: TA ratio at ripe stage of plum fruit are important parameters in determining consumer acceptance (Crisosto et al., 1995; Crisosto et al., 2007).

However, in the present study 'Angelino' plum fruit when coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) showed higher SSC (18.35%) and TA (1.06%) compared to the control and all other treatments (Fig. 6.5A and Fig 6.6A). Whilst, 'Tegan Blue' plum fruit coated with OA alone and chitosan emulsion (1.5%) loaded with OA (2.0 mM) showed the highest SSC (18.90% and 18.32% respectively) compared to all other treatments except control (Fig. 6.5B). However, an edible coating with chitosan showed significant effect on the reduction of SSC and TA value in nectarine in previous studies by slowing down the ripening and senescence process (Asgar et al., 2011; Chiabrando and Giacalone, 2013). Similarly, Han et al. (2004) explained that chitosan coating slows down the ripening and changes in the level of titratable acidity in raspberry and strawberry fruit. Similar effects of chitosan have also been reported previously on peaches (Li and Yu, 2001; Maftoonazad et al., 2008), litchi (Dong et al., 2004) and nectarine fruit (Chapter 4). Various coating treatments may have influenced SSC, TA and their ratio in plum fruit possibly through regulation of climacteric ethylene production and rate of respiration in the fruit consequently modulating the ripening process.

6.4.5. Organic acids and sugars

The main organic acid present in plum is malic acid (Le Dantec et al., 2010; Wu et al., 2011). However citric acid, tartaric acid and succinic acid have also been identified in different plum cultivars (Flores et al., 2012). From the current study it was also observed in 'Angelino' and 'Tegan Blue' plum fruit that malic acid was predominant followed by succinic acid, tartaric acid, fumaric acid and citric acid. The chitosan (1.5%) alone coating significantly ($P \leq 0.05$) resulted in higher levels of fumaric acid (2.03 mg 100 ml^{-1} FJ) and malic acid (2.50 g 100 ml^{-1} FJ) in ripe 'Angelino' plum fruit. Similarly, higher levels of citric acid (74.45 mg 100 ml^{-1} FJ) and succinic acid (0.49 g 100 ml^{-1} FJ) in ripe 'Angelino' fruit were recorded due to the combined effect of chitosan and OA. However, Palma et al. (2015) previously reported no significant effects of edible coatings on citric acid and malic acid in cactus pear fruit during storage. Similar changes were observed for levels of citric and tartaric acid in cv. Angelino plum fruit (Fig. 6.10). Yong-Hong et al. (2007) reported that there was a significant correlation between malate dehydrogenase activity and fruit malic acid content; and the activity of malic enzyme increases late in the fruit development period which decreases the content of malic acid in fruit.

However, the 'Tegan Blue' plum fruit treated with chitosan emulsion loaded with SA and SA alone showed significantly highest levels of citric acid (59.87 and 59.62mg $100ml^{-1}$ FJ) which signifies the effect of this coating treatment in reducing metabolic activities (Jitareerat et al., 2007) consequently slowing down the reduction of citric acid level in the fruit. Yong-Hong et al. (2007) reported that there was a significant correlation between malate dehydrogenase activity and fruit malic acid content; and the activity of malic enzyme increases late in the fruit development period which decreases the level of malic acid in fruit.

6.4.6. Vitamin C

In the present study, a highest levels of vitamin C was recorded in 'Angelino' plum fruit coated with SA (2.0 mM) alone and chitosan emulsion (1.5%) loaded with SA as compared to the control and all other treatments (Fig. 6.12A). Higher concentration of vitamin C (29.94 and 27.09 mg 100 ml^{-1} FW) was noted in cv. Tegan Blue when fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) and SA (2.0 mM) alone respectively compared to control and all other treatments (Fig. 6.12B). It has been previously reported that edible coatings reduce the permeability of O_2 in the fruit (Srinivasa et al., 2002) and thus delay the oxidation of ascorbic acid (Sritananan et al., 2005). Abbasi et al. (2009) also observed higher levels of vitamin C in mango fruit coated with chitosan. However, peach fruit treated with SA or OA alone showed higher level of vitamin C compared to control fruit (Tareen, 2011) which supports the experimental findings of this current study.

6.4.7. Total antioxidants

Plum fruit is a source of flavonoids and phenolic acids (Tomas-Barberan et al., 2001) with a strong antioxidant capacity (Cao et al., 1997; Vinson et al., 2001). However, great differences exist among the plum cultivars regarding their accumulation of phytochemicals and antioxidant capacity (Vizzotto et al., 2007). 'Angelino' plum fruit coated with chitosan emulsion (1.5%) alone and chitosan emulsion (1.5%) loaded with OA (2.0 mM) exhibted highest levels of total antioxidants as compared to all other treatments and control (Fig. 6.13A). Similarly, in the present study a higher level of total antioxidants (46.26 µM Trolox 100 ml^{-1} FJ) was observed in cv. Tegan Blue when coated with OA (2.0 mM) as compared to control and all other treatments (Fig. 6.13B). However, lowest level of total antioxidants (41.96 µM Trolox 100 ml^{-1} FJ) was observed in cv. Tegan Blue when fruit were coated with SA

(2.0 MM) compared to control and all treatments. However, increases in this antioxidant have been previously observed in peach fruit after postharvest OA treatment (Tareen et al., 2012). Beneficial effects of chitosan emulsion on levels of antioxidants has previously been reported for apricot (Ghasemnezhad et al., 2010), SA on peach fruit (Khademi and Ershadi, 2013), sugar apple fruit (Mo et al., 2008) and grapes (Asghari et al., 2013), and recently in our study on nectarine (Chapter 4). The exact mechanism by which chitosan, SA and OA influence levels of total antioxidants in plum fruit is yet not known and the effects of these coating treatments on the changes in the levels of various compounds like carotenoids and phenolic compounds warrants investigation.

6.4.8. Disease incidence

The use of edible coatings signifies one of the significant methods for preserving quality. Edible coatings have been traditionally used to improve food appearance and maintain quality because they are considered eco-friendly (Khwaldia et al., 2004). In the present study, percentage disease incidence was reduced with all the coating treatments in ‘Angelino’ and ‘Tegan Blue’ plum fruit compared to the control fruit (Fig. 6.14A and B). Lowest percentage of disease incidence (7.50%) was recorded when fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) as compared to control and all other treatments in ‘Tegan Blue’ plum fruit (Fig 6.14B). Chitosan emulsion loaded with the SA was the most effective treatment in reducing percentage disease incidence in cv. Tegan Blue plum fruit as compared to the application of chitosan emulsion loaded with OA and SA or chitosan alone and may be ascribed to the combined beneficial effects of both chitosan and SA.

6.5. Conclusion

Chitosan emulsion coating suppressed ethylene production during fruit ripening in both ‘Tegan Blue’ and ‘Angelino’ plums. In cultivar ‘Tegan Blue’, the fruit coated with chitosan emulsion loaded with SA exhibited lower weight loss and disease incidence, and higher levels of TA, total organic acids, total sugars, and vitamin C as compared to the uncoated fruit and fruit coated in other coatings. These results supported the hypothesis that chitosan loaded with SA is more effective than chitosan, SA or OA individual. Meanwhile, ‘Angelino’ plum fruit coated with chitosan emulsion alone exhibited suppressed ethylene production, reduced loss of

fruit firmness and disease incidence, higher SSC:TA ratio, total organic acids, sugars and total antioxidants. The results from this cultivar do not support the hypothesis and the variation in these results between both cultivars suggests a strong genotypic response to the treatments. In future, response of more plum cultivars to these coating treatments warrants to be tested.

CHAPTER 7

Impact of chitosan emulsion, salicylic acid or oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid on postharvest quality of cold stored Japanese plum (*Prunus salicina* Lindl. cv 'Angelino' and 'Tegan Blue') fruit

Summary

Chitosan, salicylic acid (SA) and oxalic acid (OA) exhibit beneficial effects on extending storage life and maintaining fruit quality. The present study was conducted to investigate the effects of chitosan emulsion, SA or OA alone and chitosan emulsion loaded with SA or OA on cold storage life and fruit quality of 'Angelino' and 'Tegan Blue' plum. Chitosan emulsion (1.5%) coating significantly ($P \leq 0.05$) suppressed mean ethylene production (49 nmol kg^{-1} h^{-1}) compared to the control (79 nmol kg^{-1} h^{-1}) and other treatments in cv. Angelino. Whilst, the chitosan emulsion (1.5%) loaded with SA (2.0 mM) suppressed ethylene production (59 nmol kg^{-1} h^{-1}) compared to control (253 nmol kg^{-1} h^{-1}) and all other treatments in 'Tegan Blue' (Table 7.2). Similarly, the chitosan (1.5%) coating alone resulted in significantly ($P \leq 0.05$) lower disease incidence (4.0%) compared to control (13.50%) in 'Angelino' plum. Chitosan emulsion (1.5%) coating resulted in higher level of titratable acidity (TA) (0.96 %), fructose (5.12 g 100 ml^{-1} FJ), glucose (4.26 g 100 ml^{-1} FJ), total sugars (9.72 g 100 ml^{-1} FJ), citric acid (49.29 mg 100 ml^{-1} FJ), malic acid (1.65 g 100 ml^{-1} FJ) and total organic acids (2.04 g 100 ml^{-1} FJ) in 'Angelino' plum fruit. Higher firmness (28.77 N), sucrose (4.94 g 100 ml^{-1} FJ), vitamin C (8.35 mg 100 ml^{-1} FJ) and reduced disease incidence (9.25%) compared to the control (17.75%) in 'Tegan Blue' fruit were recorded due to the combined effect of chitosan and SA. In conclusion, chitosan emulsion alone was more effective than control and all other treatments in reducing ethylene production, disease incidence, higher TA, total organic acids and sugars and vitamin C in cv. Angelino plum fruit. Whilst, in 'Tegan Blue' cultivar, chitosan emulsion loaded with SA were more effective in suppressing ethylene production, reducing weight loss, disease incidence, higher firmness, TA, vitamin C.

7.1. Introduction

Plums are highly perishable and undergo rapid deterioration following their harvest. Depending on the cultivar, plums may have a marketable life of 2 – 6 weeks even

when kept at 0°C (Abdi et al., 1998). Khan and Singh (2007a) claimed that limited success has been reported on extending storage life and maintaining quality of plum fruit by methods such as pre-harvest spray application of calcium (Plich et al., 2002), pre or postharvest application of inhibitors of ethylene biosynthesis such as polyamines (Serrano et al., 2003) or aminoethoxyvinylglycine (Jobling et al., 2003), and inhibitors of ethylene action such as 1-methylcyclopropene (1-MCP) (Watkins, 2006; Khan and Singh, 2007b). Khan and Singh (2007a) also claimed that limited success has been reported on extending storage life and maintaining quality of plum fruit by methods such as postharvest heat treatment (Serrano et al., 2004), edible coating (Navarro et al., 2005), controlled atmosphere and modified atmosphere (MA) storage (Turk and Ozkurt, 1994; Wang and Vestrheim, 2003) and low temperature storage (Robertson et al., 1991). The conditions (0-5 °C and 80-95% relative humidity) combined with controlled atmosphere along the supply chain delay loss of firmness, decrease weight loss and minimise incidence of diseases, but are conducive for development of chilling injury (CI) symptoms (Crisosto et al., 1999). The susceptibility of plum fruit to chilling injury is dependent on genotype, storage temperature and storage period. CI symptoms lead to deterioration of quality for consumers (Crisosto et al., 2004).

Optimal storage and transportation temperatures (7.5 °C) are used to manage CI in different cultivars of plums but result in fruit softening, over-ripening and senescence (Crisosto and Garner, 2008). Moreover, plum fruit stored for extended periods in controlled atmosphere (CA) at 7.5 °C depending upon cultivar also showed softening and 'off-flavor' particularly at low oxygen (3-5 kPa) and high CO_2 (10–15 kPa) (Crisosto and Garner, 2008). The beneficial effects of postharvest edible coatings including chitosan, salicylic acid (SA) and oxalic acid (OA) on extending postharvest life and maintenance of fruit quality in different climacteric and non-climacteric fruits including plums has been reviewed in Chapter 2 and 6. Application of chitosan coating, SA and OA alone seem to show promise for extending cold storage life and maintaining fruit quality in plum and other fruits but no information is available on the effects of chitosan emulsion loaded with SA or OA and warrants investigation. It was hypothesised that chitosan emulsion loaded with SA or OA will be more effective in extending plum fruit cold storage life compared to their individual application. Therefore, this study aimed to investigate the

influence of chitosan coating, SA and OA alone or chitosan emulsion loaded with SA or OA on rate of ethylene production and changes in various biochemical fruit quality parameters in cold stored 'Angelino' and 'Tegan Blue' Japanese plum fruit.

7.2. Materials and methods

7.2.1. Plant material

Mature 'Angelino' and 'Tegan Blue' fruit were harvested from Balingup (33° 47' S/ 115° 59' E) Western Australia. At harvest, the 'Angelino' and 'Tegan Blue' fruit had SSC = 15.77% and 16.27%, fruit firmness = 54.71 N and 56.39 N, ethylene production = 32 and 19 nmol kg^{-1} h^{-1} respectively). Following harvest, the fruit were transported to the Horticulture Research Laboratory, Curtin University, Perth, WA, and used for both experiments.

7.2.2. Treatments and experimental design

In the first experiment, coating treatments of emulsions containing chitosan (1.5%), solution of SA (2.0 mM) or OA (2.0 mM) alone or the chitosan emulsion (1.5%) loaded with SA (2.0 mM) or OA (2.0 mM) with Tween 20 (0.25%) as a surfactant were applied to 'Angelino' plum fruit. The fruit without any treatment served as a control. The fruit were allowed to dry at room temperature after the application of treatments. Subsequently, the fruit were kept at a cold temperature (0 ± 1° C and 95 ± 3% RH). Ethylene production, fruit weight loss, and other fruit quality parameters (as detailed in Chapter 6) were assessed on the fruit following 4, 6 and 8 weeks cold storage. The experiment was then repeated on 'Tegan Blue' plum fruit but the fruit were assessed after 3 and 6 weeks of cold storage. Both experiments used completely randomized design (CRD). Each included four replications and 10 fruits in each replication.

7.2.3. Determination of production of ethylene

After each cold storage period, the fruit were kept at room temperature for six hours prior to determining ethylene production. Ethylene production was determined using an ETD 300 ethylene detector (Sensor sense B.V, Nijmegen, The Netherlands) by following the method described earlier by Pranamornkith et al. (2012) and detailed in Chapter 3, Section 3.4. The ethylene was expressed as nmol kg^{-1} h^{-1}.

7.2.4. Determination of loss of fruit weight

Fruit weight loss was calculated as the percentage of fruit weight against initial weight at harvest as previously described in Chapter 3, Section 3.6.

7.2.5. Determination of fruit firmness

The firmness of fruit pulp was determined using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK) (Singh et al., 2009) as previously detailed in Chapter 3, Section 3.7. Fruit firmness was expressed as newtons (N).

7.2.6. Determination of SSC, TA and SSC:TA ratio

The percentage of SSC, TA and their ratio were determined from the plum fruit juice extracted from the pulp of ten randomly selected fruit as previously described in Chapter 3, Section 3.8.

7.2.7. Determination of individual sugars and organic acids

Individual sugars and organic acids from fruit juice were determined by using HPLC system (Waters 1525, Milford Corp., MA, USA). The detailed method has been described in Chapter 3, Section 3.9. The concentrations of sugars were expressed as g100 ml^{-1} FJ, whilst the concentrations of organic acids were calculated as g100 ml^{-1} FJ or mg100 ml^{-1} FJ.

7.2.8. Determination of vitamin C

Vitamin C concentrations were estimated using the method previously described in Chapter 3, Section 3.10 using a 6405 UV/VIS Spectrophotometer (Model 6405, Dunmow, Essex, UK). The levels of vitamin C were expressed as mg 100 ml^{-1} FJ.

7.2.9. Determination of total antioxidants

Total antioxidants were determined using 6405 UV/VIS Spectrophotometer (Model 6405, Dunmow, Essex, UK) following the modified method of Brand-William et al. (1995) and Pham (2009). The detailed method has been described in Chapter 3, Section 3.11. Total antioxidants were expressed as µM trolox equivalent antioxidant activity (TEAC) 100 ml^{-1} FJ basis.

7.2.10. Determination of disease incidence

Percentage disease incidence was determined by examining the fruit regularly and fruit were regarded as infected if a visible lesion was observed as previously detailed in Chapter 3, Section 3.13.

7.2.11. Statistical analysis

The experimental data were analysed using one-way or two-way analysis of variance (ANOVA).The detailed method has been previously explained in Chapter 3, Section 3.14.

7.3. Results

7.3.1. Ethylene production

When averaged over storage period, mean ethylene production was significantly ($P \leq 0.05$) suppressed with all the coating treatments compared to uncoated plum fruit in cv. Angelino and 'Tegan Blue' (Table 7.1 and 7.2). The 'Angelino' plum fruits coated with chitosan emulsion (1.5%) alone exhibited significantly suppressed ethylene production (49 nmol kg^{-1} h^{-1}) as compared to the control (79 nmol Kg^{-1} h^{-1}) and all other treatments (Table 7.1). The 'Tegan Blue' plum fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM SA resulted in suppressed mean ethylene production (59 nmol kg^{-1} h^{-1}) compared to control (253 nmol kg^{-1} h^{-1}) and all other treatments. When averaged over different coating treatments, mean ethylene production was highest in six-week cold stored 'Angelino' and 'Tegan Blue' plum fruit (91 and 210 nmol kg^{-1} h^{-1} respectively) as compared to other storage periods. Uncoated fruit showed the significantly highest level of mean ethylene production compared to all other treatments in both cultivars. There were significant interactions between the treatments and storage periods for ethylene production in both the plum cultivars (Table 7.1 and 7.2).

7.3.2. Weight loss

When averaged over eight weeks cold storage period, the mean weight loss was significantly lowest (7.82%) when 'Angelino' plum fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) as compared to the control (23.57%) and all other treatments (Table 7.3). Similarly, the mean weight loss was significantly lowest (8.96%) when 'Tegan Blue' plum fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) as compared to the control (16.92%) and all other treatments (Table 7.4). When averaged over different treatments, the mean weight loss was increased with the extension of cold storage period in both plum cultivars (Tables 7.3 and 7.4). The interactions between different treatments tested and cold

storage period for weight loss in both Japanese plum cultivars were found to be non-significant ($P < 0.05$).

Table 7.1. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on ethylene production during cold storage period in 'Angelino' plum fruit.

	Ethylene (nmol kg^{-1} h^{-1})				
Treatments	4 weeks	6 weeks	8 weeks	Means (T)	LSD ($P \leq 0.05$)
Control	76	126	36	79a	Treatment (T) = 3, Storage period (SP) = 2, T x SP = 6
Chitosan	48	53	48	49f	
Chitosan + SA	55	63	45	54e	
Chitosan + OA	68	90	39	65d	
Salicylic acid	73	98	39	70c	
Oxalic acid	75	115	36	75b	
Means (SP)	65b	91a	41c		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (two fruit per replication).

Table 7.2. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on ethylene production during cold storage period in 'Tegan Blue' plum fruit.

	Ethylene (nmol kg^{-1} h^{-1})			
Treatments	3 weeks	6 weeks	Means (T)	LSD ($P \leq 0.05$)
Control	115	393	253a	Treatment (T) = 64, Storage period (SP)= 37, T x SP = 91
Chitosan	71	72	71c	
Chitosan + SA	65	53	59c	
Chitosan + OA	53	121	87c	
Salicylic acid	49	288	168b	
Oxalic acid	51	320	185b	
Means (SP)	68b	210a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (two fruit per replication).

Table 7.3. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on loss of weight during cold storage period in 'Angelino' plum fruit.

	Weight loss (%)				
Treatments (T)	4 weeks	6 weeks	8 weeks	Means	LSD ($P \leq 0.05$)
Control	17.97	23.29	29.43	23.57 a	Treatment (T) = 1.51,
Chitosan	10.8	13.26	16.99	13.68 d	Storage period (SP)=
Chitosan + SA	5.63	7.93	9.91	7.82 e	1.06, T x SP = NS
Chitosan + OA	12.37	16.02	20.51	16.30 c	
Salicylic acid	18.09	22.69	27.33	22.71 a	
Oxalic acid	14.83	18.22	22.36	18.47 b	
Means (SP)	13.2c	16.90b	21.09a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 7.4. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on loss of weight during cold storage period in 'Tegan Blue' plum fruit.

	Weight loss (%)			
Treatments	3 weeks	6 weeks	Means (T)	LSD ($P \leq 0.05$)
Control	10.70	23.14	16.92a	Treatment (T) = 1.41,
Chitosan	5.70	15.59	10.65 d	Storage period (SP)=
Chitosan + SA	4.46	13.45	8.96 e	0.81, T x SP = NS
Chitosan + OA	7.22	17.25	12.23c	
Salicylic acid	8.05	19.12	13.59bc	
Oxalic acid	10.27	18.60	14.43b	
Means (SP)	7.73b	17.86a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

7.3.3. Firmness

When averaged over storage periods, chitosan emulsion (1.5%) loaded with 2.0 mM SA exhibited higher fruit firmness (44.05N and 28.77N) compared to control (38.62N and 27.20N) and all other treatments in both 'Angelino' and 'Tegan Blue' plum cultivars respectively. When averaged over treatments, mean level of firmness significantly decreased from week four (44.93N) to eight weeks after treatment (36.24N) in 'Angelino' plum fruit. Meanwhile, when averaged over treatments, mean

fruit firmness significantly decreased from week three (31.16N) to six weeks after treatment (20.65N) in 'Tegan Blue' plum fruit. The interaction between the treatments and the storage period was found to be significant for fruit firmness in cv. Tegan Blue only, but not in cv. Angelino plum (Table 7.5 and Table 7.6).

7.3.4. Soluble solids concentration (SSC)

The fruit coated with OA (2.0 mM) alone resulted in highest mean SSC (16.78% and 16.78%) when averaged over storage time in 'Angelino' and 'Tegan Blue' plum fruit compared to control (16.66% and 13.11%) respectively, as compared to all other treatments (Table 7.5 and Table 7.6). When averaged over different treatments, mean SSC significantly increased from week four (15.74%) to eight-week cold storage (16.60%) in 'Angelino' plum fruit. Meanwhile, mean level of SSC significantly decreased from week three (16.71%) to six-week cold storage (14.37%) in 'Tegan Blue' plum fruit. The interaction between the treatments and the cold storage periods was found to be significant for SSC in 'Angelino' plum fruit (Table 7.5), but not for 'Tegan Blue' plum fruit (Table 7.6).

7.3.5. Titratable acidity (TA)

When averaged over cold storage period, mean TA was highest (0.96%) in the 'Angelino' plum fruit coated with chitosan emulsion (1.5%) alone compared to control (0.90%) and all other treatments. When averaged over cold storage periods, the mean TA did not differ significantly among the treatments and control in 'Tegan Blue' plum fruit. When averaged over treatments, mean TA was significantly decreased from week four (1.04%) to eight week cold storage period (0.82%) in 'Angelino' plum fruit. and significantly decreased from week three (1.76%) to six weeks cold storage (1.20%) in 'Tegan Blue' plum fruit. The interaction between the treatments and the storage period was found to be significant for TA in 'Angelino' and 'Tegan Blue' plum fruit (Table 7.5 and Table 7.6).

7.3.6. SSC:TA ratio

When averaged over cold storage time, the fruit coated with 2.0 mM OA alone exhibited significantly highest SSC:TA ratio compared to control and all other treatments in both 'Angelino' and 'Tegan Blue' plum fruit. When averaged over treatments, mean level of SSC:TA ratio significantly increased from week four (15.63) to eighth week of cold storage (20.29) in 'Angelino' plum fruit and

significantly increased from week three (9.68) to six weeks cold storage (11.96) in 'Tegan Blue'. The interaction between the treatments and the storage period was found to be significant for SSC: TA ratio in both 'Angelino' and 'Tegan Blue' plum fruit (Table 7.5 and Table 7.6).

7.3.7. Sugars

Fructose, glucose and sucrose were quantified from 'Angelino' and 'Tegan Blue' plum fruit. Fructose is the major sugar component in both 'Angelino' and 'Tegan Blue' plum fruit.

7.3.7.1. Fructose

When averaged over cold storage period, mean levels of fructose in 'Angelino' and 'Tegan Blue' plum fruit were highest (5.12 and 5.80 g $100g^{-1}$ FJ respectively) when coated with chitosan emulsion (1.5%) as compared to the control and all other treatments (Table 7.7 and Table 7.8). Extension of cold storage period in both cultivars of plum resulted in higher mean levels of fructose when averaged over different treatments (Table 7.7 and Table 7.8). In cultivar 'Angelino', the mean concentration of fructose significantly increased from week four (4.35 g $100g^{-1}$ FJ) to eight of cold storage (5.10 g $100g^{-1}$ FJ). When averaged over all the treatments tested, the mean concentration of fructose significantly increased from week three (4.58 g $100g^{-1}$ FJ) to six weeks of cold storage (5.52 g $100g^{-1}$ FJ) in 'Tegan Blue' plum fruit. The interaction between the treatments and the ripening period was found to be significant for levels of fructose in both the cultivars.

7.3.7.2. Glucose

When averaged over different cold storage periods, mean level of glucose was highest in both 'Angelino' and 'Tegan Blue' plum fruit, which were coated with emulsion of chitosan- alone (1.5%) as compared to the control and all other treatments (Table 7.7 and 7.8). When averaged over different treatments, the six and eight-week cold stored 'Angelino' plum fruit showed higher mean concentration of glucose compared to those stored for four weeks. Similarly, 'Tegan Blue' plum fruit which were kept in cold storage for six weeks resulted in significantly higher mean level of glucose (3.40 g $100g^{-1}$ FJ) than those stored for three weeks (2.38 g $100g^{-1}$ FJ). The interaction between different treatments tested and the cold storage period

was found to be significant for levels of glucose in both cultivars (Table 7.7 and Table 7.8).

Table 7.5. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on firmness, SSC, TA and SSC:TA ratio during cold storage period in 'Angelino' plum fruit.

Firmness (N)					
Treatments	4 weeks	6 weeks	8 weeks	Means (T)	LSD ($P \leq 0.05$)
Control	42.64	43.26	29.97	38.62 b	Treatment (T) =3.67, Storage period (SP)= 2.60, T x SP = NS
Chitosan	49.91	42.42	38.80	43.71 a	
Chit + SA	50.26	44.03	37.86	44.05 a	
Chit + OA	40.02	42.41	34.58	39.00 b	
SA	45.18	39.46	39.35	41.33 ab	
OA	41.6	44.32	36.87	40.93 ab	
Means (SP)	44.93 a	42.65 a	36.24 b		
SSC (%)					
Control	15.97	17.10	16.90	16.66 ab	Treatment (T)=0.36, Storage period(SP)= 0.25, T x SP = 0.62
Chitosan	15.20	16.30	16.60	16.03 cd	
Chit + SA	15.27	16.27	15.80	15.78 d	
Chit + OA	15.77	16.37	17.00	16.38 bc	
SA	15.62	15.85	16.75	16.07 cd	
OA	16.57	17.20	16.57	16.78 a	
Means (SP)	15.74 b	16.52 a	16.60 a		
TA (%)					
Control	0.94	0.88	0.88	0.90 abc	Treatment (T)=0.06, Storage period(SP)= 0.04, T x SP = 0.11
Chitosan	1.31	0.79	0.78	0.96 a	
Chit + SA	0.90	0.93	0.82	0.89 bc	
Chit + OA	1.21	0.84	0.78	0.94 ab	
SA	1.05	0.81	0.83	0.90 abc	
OA	0.84	0.87	0.81	0.84 c	
Means (SP)	1.04 a	0.86 b	0.82 b		
SSC : TA ratio					
Control	16.92	19.35	19.17	18.48 b	Treatment (T)=0.95, Storage period(SP), 0.67, T x SP = 1.64
Chitosan	11.87	20.52	21.22	17.87 b	
Chit + SA	16.96	17.44	19.19	17.86 b	
Chit + OA	13.09	19.45	21.71	18.08 b	
SA	15.20	19.49	20.10	18.26 b	
OA	19.73	19.70	20.37	19.93 a	
Means (SP)	15.63 c	19.33 b	20.29 a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 7.6. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on firmness, SSC, TA and SSC:TA ratio during storage period in 'Tegan Blue' cultivar of plum.

Firmness (N)				
Treatments	3 weeks	6 weeks	Mean(T)	LSD ($P \leq 0.05$)
Control	33.36	21.05	27.20ab	Treatments (T) = 2.47, Storage period (SP) = 1.43, T x SP = 3.49
Chitosan	34.33	20.59	27.46ab	
Chitosan + SA	33.81	23.74	28.77a	
Chitosan + OA	30.89	20.99	25.94b	
Salicylic acid	30.83	19.01	24.92b	
Oxalic acid	23.74	18.51	21.12c	
Means (SP)	31.16a	20.65b		
SSC (%)				
Control	13.20	13.03	13.11c	Treatments (T) = 1.48, Storage period (SP) = 0.86, T x SP = NS
Chitosan	17.20	13.03	15.11b	
Chitosan + SA	16.88	13.48	15.18ab	
Chitosan + OA	17.40	15.68	16.54ab	
Salicylic acid	18.10	14.95	16.53ab	
Oxalic acid	17.48	16.08	16.78a	
Means (SP)	16.71a	14.37b		
TA (%)				
Control	2.04	1.11	1.57	Treatments (T) = NS, Storage period (SP) = 0.07, T x SP = 0.18
Chitosan	1.82	1.07	1.44	
Chitosan + SA	1.71	1.21	1.46	
Chitosan + OA	1.68	1.40	1.54	
Salicylic acid	1.66	1.19	1.43	
Oxalic acid	1.63	1.25	1.44	
Means (SP)	1.76a	1.20b		
SSC : TA ratio				
Control	6.48	11.64	9.06c	Treatments (T) = 0.92, Storage period (SP) = 0.53, T x SP = 1.30
Chitosan	9.47	12.25	10.86b	
Chitosan + SA	9.90	11.21	10.55b	
Chitosan + OA	10.47	11.21	10.84ab	
Salicylic acid	10.99	12.58	11.78a	
Oxalic acid	10.76	12.90	11.83a	
Means (SP)	9.68b	11.96a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

7.3.7.3. Sucrose

'Angelino' plum fruit coated with chitosan emulsion (1.5%) loaded with (2.0 mM) SA exhibited highest mean levels of sucrose (407.04 mg $100g^{-1}$ FJ) averaged over cold storage period, followed by the fruit coated with 2.0 mM SA (380.24 mg $100g^{-1}$ FJ) and chitosan emulsion (1.5%) loaded with 2.0 mM OA (339.12 mg $100g^{-1}$ FJ) (Table. 7.7). Mean levels of sucrose were lowest in 'Angelino' plum fruit which were coated with 2.0 mM OA alone (287.16 mg $100g^{-1}$ FJ). When averaged over cold storage time, mean sucrose level was highest (4.94 g $100g^{-1}$ FJ) in the 'Tegan Blue' plum fruit which were coated with chitosan emulsion (1.5%) loaded with (2.0 mM) SA compared to control and all other treatments (Table 7.8). Mean concentration of sucrose was lowest (3.65 g $100g^{-1}$ FJ) in the 'Tegan Blue' plum fruit which was coated with OA (2.0 mM) alone as compared to all other treatments and control. The mean concentration of sucrose significantly decreased in eight weeks cold stored 'Angelino' plum fruit (281.4 mg $100g^{-1}$ FJ) as compared to those which were stored for four weeks (463.6 mg $100g^{-1}$ FJ). Similarly, mean concentrations of sucrose when averaged over treatments, decreased significantly in the 'Tegan Blue' plum fruit stored for three weeks (5.24 g $100g^{-1}$ FJ) to six weeks cold storage (3.39 g $100g^{-1}$ FJ). The interaction between the treatments and the cold storage period for sucrose concentration was found to be significant only for 'Angelino' plum fruit.

7.3.7.4. Total sugars

'Angelino' plum fruit coated with chitosan emulsion (1.5%) alone exhibited highest mean levels of total sugars (9.72 g $100g^{-1}$ FJ) compared to all other treatments and control (Table 7.7). The fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM OA showed lowest levels of total sugars (8.63 g $100g^{-1}$ FJ) compared with all other treatments and control (Table 7.7). When averaged over cold storage period, the mean concentrations of total sugars was highest (14.02 g $100g^{-1}$ FJ) in the 'Tegan Blue' plum fruit which were coated with chitosan emulsion (1.5%) alone as compared with control and all other treatments (Table 7.8). Mean concentration of total sugars was lowest in 'Tegan Blue' plum fruit (10.87 g $100g^{-1}$ FJ) which were treated with 2.0 mM SA compared to all other treatments and control. Mean concentration of total sugars increased significantly in both 'Angelino' and 'Tegan Blue' plum fruit with the extension of cold storage periods. The interactions between

different treatments and cold storage periods for total sugars in the fruit of both plum cultivars were found to be significant (Table 7.7 and 7.8).

Table 7.7. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of fructose, glucose, sucrose and total sugars during cold storage period in the juice of 'Angelino' plum fruit.

Fructose (g $100g^{-1}$ FJ^{-1})					
Treatments	4 weeks	6 weeks	8 weeks	Means (T)	LSD ($P \leq 0.05$)
Control	4.38	4.89	5.52	4.93ab	Treatment (T)= 0.21, Storage period (SP)= 0.15, T x SP = 0.37
Chitosan	4.17	5.71	5.48	5.12a	
Chit + SA	4.66	5.04	5.11	4.94ab	
Chit + OA	4.34	4.18	4.86	4.46c	
Salicylic acid	4.34	5.15	4.70	4.76b	
Oxalic acid	4.18	4.45	4.85	4.50c	
Means (SP)	4.35c	4.91b	5.10a		
Glucose (g $100g^{-1}$ FJ^{-1})					
Control	3.95	4.11	4.67	4.24a	Treatment (T)= 0.19, Storage period (SP)= 0.13, T x SP = 0.33
Chitosan	3.67	4.55	4.56	4.26a	
Chit + SA	3.92	4.32	4.05	4.10ab	
Chit + OA	3.76	3.68	4.05	3.83c	
Salicylic acid	3.81	4.55	4.20	4.19a	
Oxalic acid	3.64	3.97	4.20	3.94bc	
Means (SP)	3.79b	4.20a	4.29a		
Sucrose (mg 100g-1 FJ-1)					
Control	435.20	234.05	249.50	306.2c	Treatment (T)= 49.51, Storage period (SP)= 35.01, T x SP = 85.76
Chitosan	463.40	289.57	257.25	336.7bc	
Chit + SA	586.92	252.45	381.75	407.0a	
Chit + OA	468.25	298.35	250.75	339.1bc	
Salicylic acid	465.72	371.25	303.75	380.2ab	
Oxalic acid	362.25	253.62	245.62	287.2c	
Means (SP)	463.6a	283.2b	281.4b		
Total sugars (g $100g^{-1}$ FJ^{-1})					
Control	8.76	9.23	10.44	9.48a	Treatment (T)= 0.37, Storage period (SP)= 0.26, T x SP = 0.63
Chitosan	8.30	10.55	10.30	9.72a	
Chit + SA	9.18	9.62	9.55	9.45a	
Chit + OA	8.57	8.16	9.16	8.63b	
Salicylic acid	8.63	10.07	9.29	9.33a	
Oxalic acid	8.18	8.67	9.30	8.72b	
Means (SP)	8.60c	9.39b	9.67a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 7.8. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of fructose, glucose, sucrose and total sugars in the juice of 'Tegan Blue' plum fruit during cold storage period.

Fructose (g $100g^{-1}$ FJ)				
Treatments	3 weeks	6 weeks	Mean(T)	LSD ($P \leq 0.05$)
Control	3.44	6.53	4.98 ab	Treatments (T) = 0.80,
Chitosan	5.69	5.90	5.80 a	Storage period (SP) = 0.46,
Chitosan + SA	5.35	4.62	4.99 ab	T x SP = 1.13
Chitosan + OA	4.75	5.92	5.33 ab	
Salicylic acid	4.21	4.89	4.55 b	
Oxalic acid	4.07	5.27	4.67 b	
Means (SP)	4.58 b	5.52 a		
	Glucose (g $100g^{-1}$ FJ)			LSD ($P \leq 0.05$)
Control	1.80	4.21	3.00 b	Treatments (T) = 0.32,
Chitosan	3.37	3.44	3.41 a	Storage period (SP) =
Chitosan + SA	2.45	2.81	2.63 c	0.18, T x SP = 0.45
Chitosan + OA	2.31	3.21	2.76 bc	
Salicylic acid	2.15	3.16	2.65 c	
Oxalic acid	2.18	3.57	2.87 bc	
Means (SP)	2.38 b	3.40 a		
	Sucrose (g $100g^{-1}$ FJ)			LSD ($P \leq 0.05$)
Control	4.86	2.97	3.92 bc	Treatments (T) = 0.90,
Chitosan	6.06	3.57	4.82 ab	Storage period (SP) =
Chitosan + SA	5.88	4.00	4.94 a	0.52, T x SP = NS
Chitosan + OA	5.14	4.63	4.88 a	
Salicylic acid	4.88	2.46	3.67 c	
Oxalic acid	4.61	2.69	3.65 c	
Means (SP)	5.24 a	3.39 b		
	Total sugars (g $100g^{-1}$ FJ)			LSD ($P \leq 0.05$)
Control	10.10	13.71	11.90 bc	Treatments (T) = 1.72,
Chitosan	15.13	12.91	14.02 a	Storage period (SP) = NS,
Chitosan + SA	13.69	11.44	12.57 abc	T x SP = 2.43
Chitosan + OA	12.20	13.76	12.98 ab	
Salicylic acid	11.24	10.51	10.87 c	
Oxalic acid	10.86	11.53	11.20 bc	
Means (SP)	12.20	12.31		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

7.3.8. Organic acids

Five organic acids were detected in plum fruit namely citric acid, malic acid, fumaric acid, tartaric acid and succinic acid. Malic acid was a major organic acid in both 'Angelino' and 'Tegan Blue' plum fruit (Tables 7.9 and 7.10).

7.3.8.1. Citric acid

'Angelino' plum fruit coated with chitosan emulsion (1.5%) alone showed the highest mean concentration of citric acid (49.29 mg $100g^{-1}$ FJ) averaged over cold storage period compared to the control (45.40 mg $100g^{-1}$ FJ) and all other treatments (Table. 7.9). When averaged over different treatments, the mean levels of citric acid were significantly lowest (38.04 mg $100g^{-1}$ FJ) in eight weeks cold stored fruit compared to those stored for four and six weeks. Whilst, in cultivar 'Tegan Blue', the mean levels of citric acid were not significantly influenced by the treatments and cold storage period (Table 7.10). The interaction between different treatments and cold storage period for citric acid was found to be significant in 'Angelino' and 'Tegan Blue' plum fruit.

7.3.8.2. Malic acid

When averaged over cold storage time, 'Angelino' and 'Tegan Blue' plum fruit which were coated with chitosan emulsion (1.5%) resulted in highest level of malic acid (1.65 g $100g^{-1}$ FJ and 4.07 g $100g^{-1}$ FJ) as compared to the control (1.36 g $100g^{-1}$ FJ and 3.12 g $100g^{-1}$ FJ) and all other treatments respectively (Table 7.9 and Table 7.10). When averaged over different treatments, mean levels of malic acid declined with the extension of cold storage period in both the cultivars. The interaction between different treatments and the cold storage period for levels of malic acid in 'Angelino' plum fruit was found to be significant but not for cultivar 'Tegan Blue'.

7.3.8.3. Tartaric acid

When averaged over cold storage time, mean levels of tartaric acid were not affected significantly by any treatment in 'Angelino' and 'Tegan Blue' plum fruit (Table 7.9 and Table 7.10). Averaged over different treatments, mean level of tartaric acid increased significantly in eight weeks cold stored 'Angelino' plum fruit (1.59 mg $100g^{-1}$ FJ) as compared to those which were stored for four and six weeks. Similarly, 'Tegan Blue' plum fruit stored for six weeks exhibited significantly higher levels of mean tartaric acid (3.60 mg $100g^{-1}$ FJ) than those which were stored for three weeks (1.56 mg $100g^{-1}$ FJ). The interaction between different treatments and cold storage period was found to be non-significant for levels of tartaric acid in 'Angelino' plum fruit but significant for 'Tegan Blue' plum fruit.

7.3.8.4. Fumaric acid

When averaged over cold storage time, all the treatments have reduced the mean levels of fumaric acid in the fruit of both cultivars but the effects of treatments were significant on 'Tegan Blue' plum fruit (Table 7.10). The effects of cold storage period on the levels of fumaric acid in the 'Angelino' fruit were found to be non-significant. When averaged over treatments, mean levels of fumaric acid were significantly higher (2.05 mg $100g^{-1}$ FJ) in six weeks cold stored 'Tegan Blue' fruit compared to those stored for three weeks (1.19 mg $100g^{-1}$ FJ). The interaction between different treatments and cold storage period for levels of fumaric acid in 'Tegan Blue' plum fruit was found to be significant but not significant for 'Angelino' (Table 7.9).

7.3.8.5. Succinic acid

When averaged over cold storage time, mean concentration of succinic acid in 'Angelino' plum fruit was highest (345.0 mg $100g^{-1}$ FJ) in those coated with chitosan emulsion (1.5%) loaded with 2.0 mM OA compared to control (325.6 mg $100g^{-1}$ FJ) and all other treatments (Table 7.9). When averaged over cold storage time, mean concentration of succinic acid in 'Tegan Blue' plum fruit was highest (474.25 mg $100g^{-1}$ FJ) in those coated with chitosan emulsion (1.5%) compared to control (378.50 mg $100g^{-1}$ FJ) and all other treatments (Table 7.10). When averaged over different treatments, the mean concentration of succinic acid decreased (0.88-fold) significantly from four to eight weeks cold stored 'Angelino' plum fruit. When averaged over different treatments, mean concentration of succinic acid decreased (0.92-fold) significantly from three to six weeks cold stored 'Tegan Blue' plum fruit. A significant interaction for levels of succinic acid between different treatments and cold storage time was recorded in 'Angelino' and 'Tegan Blue' plum fruit (Table 7.9 and 7.10).

7.3.8.6. Total organic acids

'Angelino' plum fruit exhibited significantly highest mean levels of total organic acids (2.04 g $100g^{-1}$ FJ) when coated with chitosan emulsion (1.5%) alone compared with control (1.73 g $100g^{-1}$ FJ) and all other treatments except fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM OA (Table 7.9).When averaged over cold storage time, the 'Tegan Blue' plum fruit coated with chitosan emulsion (1.5%) exhibited highest level of total organic acids (4.61g $100g^{-1}$ FJ) compared to control

(3.56 g $100g^{-1}$ FJ) and all other treatments (Table 7.10). When averaged over different treatments, mean level of total organic acids decreased (0.74-fold) significantly in eight weeks cold stored 'Angelino' plum fruit compared to four weeks. When averaged over different treatments, mean level of total organic acids decreased (0.88-fold) significantly in six weeks cold stored fruit compared to three week cold stored 'Tegan Blue' plum fruit. The interaction between different treatments and cold storage period was found to be significant for levels of total organic acid in 'Angelino' plum fruit but not for 'Tegan Blue'.

7.3.9. Vitamin C

All the coating treatments significantly reduced the levels of mean vitamin C in 'Angelino' plum fruit compared to the uncoated control fruit (Table 7.11). Meanwhile, when averaged over cold storage period, mean concentration of vitamin C in 'Tegan Blue' plum fruit was significantly highest (8.35 mg $100ml^{-1}$ FJ) in those coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) compared to control and all other treatments (Table 7.12). When averaged over all the treatments, mean levels of vitamin C were significantly reduced in 'Angelino' plum fruit with the extension of cold storage period. When averaged over cold storage time, mean concentration of vitamin C significantly decreased (0.70-fold) in six weeks cold stored 'Tegan Blue' plum fruit compared to three weeks (Table 7.12). The interaction between different treatments and cold storage period was not significant for levels of vitamin C in both 'Angelino' and 'Tegan Blue' plum fruit.

7.3.10. Total antioxidants

When averaged over cold storage time, 'Angelino' plum fruit treated with 2.0 mM SA exhibited significantly highest mean level of total antioxidants (47.47 μM Trolox 100 ml^{-1} FJ) compared to control and all other treatments (Table 7.13). In cultivar 'Tegan Blue', mean level of total antioxidants was significantly highest (43.79 μM Trolox 100 ml^{-1} FJ) compared to control and all other treatments except chitosan emulsion (1.5%) loaded with OA (2.0 mM) (Table 7.14). When averaged over different treatments, the mean level of total antioxidants significantly increased in eight week cold stored 'Angelino' plum fruit as compared to those stored for four and six weeks but the effect was reversed in 'Tegan Blue' plums. The interaction between different treatments and the cold storage period for levels of total

antioxidants was found to be significant only in 'Tegan Blue' plum fruit but not in 'Angelino' plum fruit.

Table 7.9. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of citric acid, malic acid, tartaric acid, fumaric acid, succinic acid and total organic acids in the juice of 'Angelino' cultivar of plum during cold storage period.

Citric acid (mg $100g^{-1}$ FJ)					
Treatments	4 weeks	6 weeks	8 weeks	Means (T)	LSD ($P \leq 0.05$)
Control	45.53	51.15	39.53	45.40 c	Treatment (T) = 2.24,
Chitosan	52.10	55.30	40.46	49.29 a	Storage period (SP)
Chit+SA	56.35	50.95	37.08	48.13 ab	= 1.58, T X SP = 3.88
Chit+OA	53.83	49.20	37.25	46.76 bc	
SA	51.80	54.35	36.73	47.63 abc	
OA	51.95	49.75	37.18	46.29 bc	
Means(SP)	51.93 a	51.78 a	38.04 b		
Malic acid (g $100g^{-1}$ FJ)					
Control	1.50	1.41	1.16	1.36 d	Treatment (T) = 0.08,
Chitosan	1.79	1.52	1.64	1.65 a	Storage period (SP)
Chit+SA	1.91	1.36	1.12	1.46 c	= 0.06, T X SP = 0.14
Chit+OA	1.87	1.74	1.22	1.61 ab	
SA	1.77	1.71	1.16	1.55 bc	
OA	1.69	1.58	1.19	1.49 c	
Means(SP)	1.75 a	1.55 b	1.25 c		
Tartaric acid (mg $100g^{-1}$ FJ)					
Control	1.20	1.20	1.60	1.33	Treatment (T) = NS,
Chitosan	1.20	1.20	1.53	1.31	Storage period (SP)
Chit+SA	1.25	1.20	1.60	1.35	= 0.02, T X SP = NS
Chit+OA	1.20	1.20	1.60	1.33	
SA	1.20	1.20	1.60	1.33	
OA	1.20	1.20	1.60	1.33	
Means(SP)	1.21 b	1.20 b	1.59 a		
Fumaric acid (mg $100g^{-1}$ FJ)					
Control	1.37	0.87	0.90	1.05	Treatment (T) = NS,
Chitosan	0.90	0.90	0.90	0.90	Storage period (SP)
Chit+SA	0.92	0.87	0.99	0.93	= NS, T X SP = NS
Chit+OA	0.90	0.87	0.90	0.89	
SA	0.90	0.85	0.90	0.88	
OA	0.87	0.85	0.90	0.87	
Means(SP)	0.98	0.87	0.92		
Succinic acid (mg $100g^{-1}$ FJ)					
Control	312.38	370.10	294.28	325.6 bc	Treatment (T)=15.93,
Chitosan	334.60	381.88	309.62	342.0 ab	Storage period (SP)
Chit+SA	342.60	344.48	279.88	322.3 c	=11.26, T X SP=27.6
Chit+OA	379.43	335.93	319.75	345.0 a	
SA	344.08	375.75	295.57	338.5 abc	
OA	336.82	328.45	301.15	322.1 c	
Means(SP)	341.7 b	356.1 a	300.0 c		

			Total organic acids (g 100g^{-1} FJ)		
Control	1.86	1.83	1.50	1.73 e	Treatment (T) = 0.09,
Chitosan	2.18	1.96	1.99	2.04 a	Storage period (SP)
Chit+SA	2.31	1.75	1.44	1.83 d	= 0.07, T X SP=0.16
Chit+OA	2.30	2.13	1.58	2.00 ab	
SA	2.16	2.14	1.50	1.93 bc	
OA	2.08	1.96	1.53	1.86 cd	
Means(SP)	2.15 a	1.96 b	1.59 c		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 7.10. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on levels of citric acid, malic acid, tartaric acid, fumaric acid, succinic acid and total organic acids in the juice of 'Tegan Blue' cultivar of plum during cold storage period.

		Citric acid (mg 100g^{-1} FJ)		
Treatments	3 weeks	6 weeks	Mean(T)	LSD ($P \leq 0.05$)
Control	58.97	67.20	63.09	Treatments (T) = NS,
Chitosan	72.50	60.85	66.67	Storage period (SP) = NS,
Chitosan + SA	66.40	61.07	63.73	T x SP = 11.86
Chitosan + OA	59.42	65.52	62.47	
Salicylic acid	61.10	58.75	59.92	
Oxalic acid	59.95	63.85	61.90	
Means (SP)	63.06	62.87		
		Malic acid (g 100g^{-1} FJ)		LSD ($P \leq 0.05$)
Control	3.00	3.24	3.12 b	Treatments (T) = 0.55,
Chitosan	4.71	3.43	4.07 a	Storage period (SP) = 0.32,
Chitosan + SA	4.06	3.12	3.59 ab	T x SP = NS
Chitosan + OA	3.63	3.60	3.62 ab	
Salicylic acid	3.33	2.94	3.14 b	
Oxalic acid	3.17	2.87	3.02 b	
Means (SP)	3.65 a	3.20 b		
		Tartaric acid (mg 100g^{-1} FJ)		LSD ($P \leq 0.05$)
Control	1.50	3.60	2.55	Treatments (T) = NS,
Chitosan	1.60	3.62	2.61	Storage period (SP) = 0.04,
Chitosan + SA	1.70	3.55	2.62	T x SP = 0.09
Chitosan + OA	1.50	3.60	2.55	
Salicylic acid	1.50	3.62	2.56	
Oxalic acid	1.57	3.65	2.61	
Means (SP)	1.56 b	3.60 a		

	Fumaric acid (mg 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	1.20	2.12	1.66 a	Treatments (T) = 0.04,
Chitosan	1.15	2.05	1.60 c	Storage period (SP) = 0.03,
Chitosan + SA	1.20	2.02	1.61 bc	T x SP = 0.06
Chitosan + OA	1.22	2.02	1.62 abc	
Salicylic acid	1.20	2.00	1.60 bc	
Oxalic acid	1.20	2.10	1.65 ab	
Means (SP)	1.19 b	2.05 a		
	Succinic acid (mg 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	332.60	424.40	378.50 b	Treatments (T) = 57.46,
Chitosan	534.00	414.50	474.25 a	Storage period (SP) = 33.17,
Chitosan + SA	470.30	378.50	424.40 ab	T x SP = 81.26
Chitosan + OA	440.00	417.30	428.65 ab	
Salicylic acid	396.40	342.90	369.65 b	
Oxalic acid	371.70	369.10	370.40 b	
Means (SP)	424.2 a	391.1 b		
	Total organic acids (g 100g^{-1} FJ)			LSD ($P \leq 0.05$)
Control	3.39	3.74	3.56 b	Treatments (T) = 0.61,
Chitosan	5.32	3.91	4.61 a	Storage period (SP) = 0.35,
Chitosan + SA	4.60	3.57	4.08 ab	T x SP = NS
Chitosan + OA	4.13	4.09	4.11 ab	
Salicylic acid	3.79	3.35	3.57 b	
Oxalic acid	3.60	3.31	3.45 b	
Means (SP)	4.14 a	3.66 b		

Any two means within a column and a row followed by different letters are significantly different using LSD at P ≤ 0.05. NS = not significant, n = four replicates (ten fruit per replication).

Table 7.11. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on level of vitamin C in the juice of 'Angelino' cultivar of plum during cold storage period.

	Vitamin C (mg 100 ml^{-1} FJ)				
Treatments	4 weeks	6 weeks	8 weeks	Means (T)	LSD ($P \leq 0.05$)
Control	31.12	33.75	31.06	31.98 a	Treatment (T) = 3.31,
Chitosan	26.85	27.47	25.10	26.47 b	Storage period (SP) =
Chitosan + SA	26.04	24.65	26.98	25.89 b	NS, T x SP = NS
Chitosan + OA	29.18	28.60	22.22	26.67 b	
Salicylic acid	28.66	24.75	23.32	25.58 b	
Oxalic acid	27.01	22.42	25.49	24.97 b	
Means (SP)	28.15	26.94	25.70		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 7.12. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on level of vitamin C in the juice of 'Tegan Blue' cultivar of plum during cold storage period.

Vitamin C (mg 100 ml^{-1} FJ)				
Treatments	3 weeks	6 weeks	Means (T)	LSD ($P \leq 0.05$)
Control	4.63	1.38	3.00 e	Treatment (T) = 0.45,
Chitosan	7.04	4.97	6.00 c	Storage period (SP)=
Chitosan + salicylic acid	9.08	7.62	8.35 a	0.25, T x SP = NS
Chitosan + oxalic acid	6.46	4.61	5.53 cd	
Salicylic acid	8.01	6.43	7.22 b	
Oxalic acid	5.84	3.61	4.73 d	
Means (SP)	6.84 a	4.77 b		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 7.13. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA acid on level of total antioxidants in the juice of 'Angelino' cultivar of plum during storage period.

Total antioxidants (µM Trolox 100 ml^{-1} FJ)					
Treatments	4 weeks	6 weeks	8 weeks	Means (T)	LSD ($P \leq 0.05$)
Control	41.53	41.96	43.03	42.17 e	Treatment (T) = 0.29,
Chitosan	44.40	45.02	45.44	44.95 d	Storage period (SP) =
Chitosan + SA	45.14	46.19	46.96	46.10 c	0.20, T x SP = NS
Chitosan + OA	45.94	47.06	47.70	46.90 b	
Salicylic acid	46.88	47.38	48.15	47.47 a	
Oxalic acid	40.59	41.01	42.41	41.34 f	
Means (SP)	44.08 c	44.77 b	45.61 a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 7.14. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on level of total antioxidants in the juice of 'Tegan Blue' cultivar of plum during storage period.

Total antioxidants (µM Trolox 100 ml^{-1} FJ)				
Treatments	3 weeks	6 weeks	Means (T)	LSD ($P \leq 0.05$)
Control	42.43	41.71	42.07b	Treatment (T) = 0.75,
Chitosan	42.08	41.91	42.00b	Storage period (SP)=
Chitosan + salicylic acid	42.75	42.11	42.43b	0.43, T x SP = 1.06
Chitosan + oxalic acid	44.47	42.73	43.60a	
Salicylic acid	44.40	43.18	43.79a	
Oxalic acid	43.80	40.99	42.39b	
Means (SP)	43.32a	42.10b		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

7.3.11. Disease incidence

No disease incidence was recorded in 4-6 weeks cold stored 'Angelino' plum fruit irrespective of the treatment. 'Angelino' plum fruit coated with emulsion of chitosan (1.5%) alone exhibited significantly lowest percentage disease incidence (4.0%) after eight weeks of cold storage as compared to the control (13.5%) and all other treatments except the fruit treated with OA (Fig. 7.1A). The disease incidence as noticed on only six weeks cold stored 'Tegan Blue' plum fruit. The 'Tegan Blue' plum fruit coated with emulsion of chitosan (1.5%) loaded with SA exhibited significantly lowest percentage disease incidence (9.25%) following six weeks cold storage as compared to the control (17.75%) and all other treatments (Fig. 7.1B). The interaction between different treatments and cold storage period was found to be significant for levels of disease incidence in both 'Angelino' and 'Tegan Blue' plum fruit.

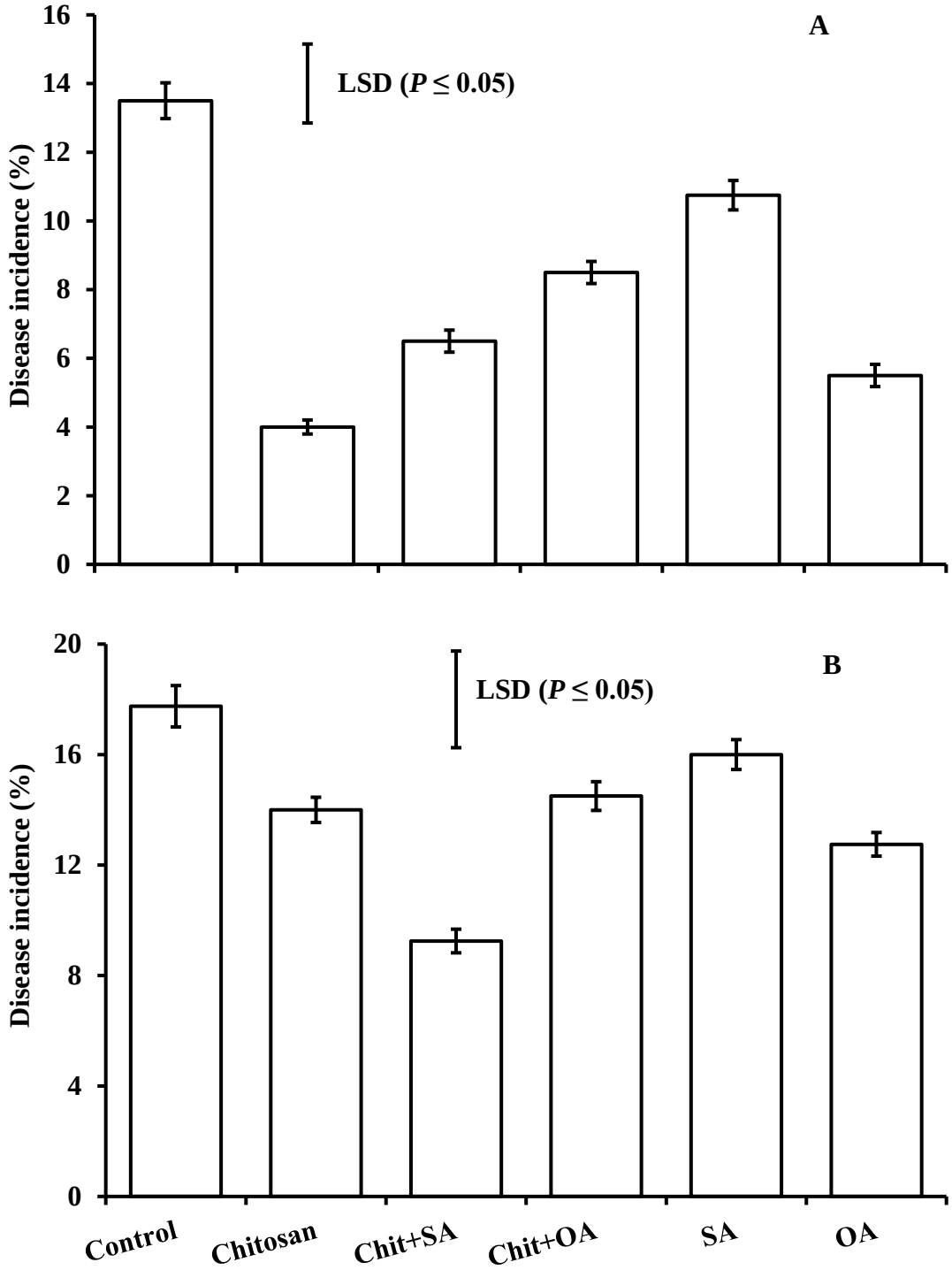


Figure 7.1. A and B. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on percentage disease incidence in (A) 'Angelino' and (B) 'Tegan Blue' cultivars of plum during cold storage period. Vertical bars represent SE, n = four replicates (ten fruit per replication).

7.4. Discussion

In this experiment, the effects of postharvest application of chitosan emulsion, SA, OA alone and chitosan emulsion loaded with SA, OA on ethylene production, extension of cold storage life and maintenance of fruit quality of 'Angelino' and 'Tegan Blue' Japanese plums have been investigated to address the second objective of this research.

7.4.1. Ethylene production

Chitosan emulsion (1.5%) coating significantly ($P \leq 0.05$) suppressed climacteric ethylene production compared to the control and other treatments in 'Angelino' plum fruit during cold storage period (Table 7.1). 'Tegan Blue' plum fruit coated with chitosan alone, chitosan emulsion (1.5%) loaded with 2.0 mM SA followed by chitosan emulsion (1.5%) loaded with 2.0 mM OA suppressed climacteric ethylene production (Table 7.2). The possible mode of reduction of ethylene production during fruit ripening in the fruit coated with chitosan, OA and SA alone or in combination with chitosan emulsion has been discussed in the Chapter 6, Section 6.4.1. The response of both 'Angelino' and 'Tegan Blue' Japanese plum cultivars differed with the treatments tested in supressing ethylene production in the fruit and may be ascribed to the genotypic differences between both cultivars. Earlier, variation in climacteric ethylene production in the fruit of different cultivars of Japanese plum has been reported (Abdi et al., 1998; Khan and Singh, 2007a; Singh et al., 2012).

7.4.2. Weight loss

Fruit weight loss in the postharvest phase is coupled with moisture evaporation from the fruit surface and respiration rate. In the present study, coating of chitosan emulsion loaded with SA and chitosan emulsion alone reduced the loss of weight in both 'Angelino' and 'Tegan Blue' cultivars of plums. The fruit coated with chitosan loaded with SA exhibited significantly least weight loss compared to the control and other treatments in both 'Angelino' and 'Tegan Blue' plum fruit (Table 7.3 and Table 7.4). The reduction in loss of weight in both 'Angelino' and 'Tegan Blue' fruit coated with chitosan emulsion loaded with SA has been explained earlier in Chapter 6, Section 6.4.2). The beneficial effects of SA on reduction of weight loss have been reported for plum fruit in cold storage (Davaryneiad et al., 2013). Similarly, least significant weight loss was recorded in chitosan emulsion coated fruit in both 'Honey

Fire' and 'Bright Pearl' nectarine compared to the control as reported in Chapter 4. Chitosan emulsion loaded with SA was the most effective treatment in reducing weight loss in both cultivars as compared to all other treatments and may be ascribed to the combined beneficial effects of both chitosan and SA.

7.4.3. Firmness

Rapid fruit softening and ripening during postharvest phase is one of the critical factors contributing to the short postharvest life in plum. The fruit firmness was found to be higher in both 'Angelino' and 'Tegan Blue' cultivars of plum when fruit coated with chitosan emulsion loaded with SA as compared to control and all other treatments and may possibly be attributed to the decreased ethylene production (Table. 7.5 and Table 7.6). Possibly, suppression of ethylene production in plum fruit which were coated with chitosan, OA and SA may have contributed to the delayed loss of fruit firmness as has been explained earlier in Chapter 6, Section 6.4.3 for plum fruit. Higher mean level of firmness in 'Angelino' (44.05 N) than the 'Tegan Blue' (28.77 N) cold stored plum fruit coated with the chitosan emulsion loaded with SA suggests that fruit firmness is also influenced by genotype and a similar trend has also been noted in Chapter 6, Section 6.4.3 for plum fruit.

7.4.4. SSC, TA and SSC: TA ratio

In the present study, 'Angelino' and 'Tegan Blue' plum fruits when coated with OA (2.0 mM) showed higher SSC (16.78% and 16.78%) and SSC: TA ratio (19.93% and 11.83%) respectively compared to the control and all other treatments (Table 7.5 and Table 7.6). The fruit coated with chitosan emulsion exhibited higher level of TA compared to control and all other treatments in 'Angelino' plum fruit. Whilst, the treatments did not show a significant effect on TA in 'Tegan Blue' plum fruit (Table 7.5 and Table 7.6). The changes in SCC, TA and their ratio in the cold stored plum fruits coated with chitosan, OA or SA have been discussed in the Chapter 6, Section 6.4.4.

7.4.5. Organic acids and sugars

Amongst different organic acids in the 'Angelino' and 'Tegan Blue' plum fruit, malic acid was predominant followed by succinic acid, citric acid, tartaric acid and fumaric acid as also noted in Chapter 6, Section 6.4.5. The chitosan (1.5%) alone coating resulted in higher level of malic acid (1.65 g 100 ml^{-1} FJ) and total organic

acids (2.04 g 100 ml^{-1} FJ) in 'Angelino' plum fruit. Similarly, higher level of malic acid (4.07 g 100 ml^{-1} FJ) and succinic acid (474.25 mg 100 ml^{-1} FJ) were recorded in 'Tegan Blue' fruit coated with chitosan emulsion alone.

Fructose was a major sugar in 'Angelino' plum fruit followed by glucose and sucrose (Table 7.7). Meanwhile, fructose was the major sugar component found in 'Tegan Blue' plum fruit. Regulation of levels of organic acids and sugars in stored plum fruit due to the treatment of chitosan, OA and SA has been discussed in Chapter 6, Section 6.4.5.

7.4.6. Vitamin C

In the present study, significantly higher concentration of vitamin C (8.35 mg 100 ml^{-1} FW) was noted in cv. Tegan Blue when fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) compared to control and all other treatments (Table 7.12). Edible coatings restrict the permeability of O_2 and CO_2 into the fruit consequently reducing oxidation of ascorbic acid (Sritananan et al., 2005). The beneficial effects of chitosan coating, OA and SA in maintaining higher levels of vitamin C in stored plum fruit has also been discussed in detail in Chapter 6, Section 6.4.6. The variation in the vitamin C levels due to different treatments differs in both cultivars of plums tested and hence seems to be genotype dependent.

7.4.7. Total antioxidants

Higher levels of total antioxidants (47.47 and 43.79 μM Trolox 100 ml^{-1} FJ) were noted in both 'Angelino' and 'Tegan Blue' cultivars respectively when coated with SA (2.0 mM) as compared to control and all other treatments (Table 7.13 and Table 7.14). Changes in the levels of antioxidants in stored plum fruit with chitosan, SA and OA have been reported in Chapter 6, Section 6.4.7. The precise mechanism of chitosan, SA and OA in regulating total antioxidants in cold stored plum fruit is not known and hence is worth examining.

7.4.8. Disease incidence

In the present study, lowest percentage of disease incidence (4%) was recorded when fruit were coated with chitosan emulsion (1.5%) alone as compared to control and all other treatments in 'Angelino' plum fruit cold stored for eight weeks (Fig. 7.1A). Meanwhile, lowest percentage of disease incidence (9.25%) was recorded when fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) as compared to

control and all other treatments in 'Tegan Blue' plum fruit cold stored for six weeks (Fig. 7.1B). Chitosan emulsion loaded with SA was a most effective treatment in reducing percentage disease incidence in cv. Tegan Blue plum fruit as compared to the application of chitosan emulsion loaded with OA and SA or chitosan alone and may be ascribed to the combined beneficial effects of both chitosan and SA. Various mechanisms for reduction of incidence of disease in stored plum fruit with the treatments of chitosan, OA and SA have been discussed in Chapter 6, Section 6.4.8.

7.5. Conclusion

Chitosan emulsion (1.5%) coating significantly suppressed mean ethylene production compared to the control and other treatments in 'Angelino' plum fruit. Chitosan emulsion (1.5%) loaded with SA suppressed mean ethylene production in 'Tegan Blue' plum fruit. Similarly, the chitosan (1.5%) coating alone resulted in a significantly ($P \leq 0.05$) lower loss of weight and disease incidence in 'Angelino' plum fruit. Whilst, chitosan coating recorded higher levels of TA, fructose, glucose, total sugars, citric acid, malic acid and total organic acids in 'Angelino' plum fruit. Higher levels of firmness, sucrose and vitamin C and reduced disease incidence compared to control in 'Tegan Blue' fruit were recorded due to the combined effect of chitosan and SA. In conclusion, the hypothesis tested whether chitosan loaded with SA or OA is more effective in reducing ethylene production, weight loss and disease incidence, higher levels of TA, sucrose, tartaric acid and vitamin C in cold conditions compared to the application of chitosan, SA or OA alone was confirmed in 'Tegan Blue' plum cultivar but not in 'Angelino'.

CHAPTER 8

Effects of chitosan emulsion, salicylic acid or oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid on postharvest quality of sweet orange (cv. Midknight Valencia) fruit stored at different low temperatures

Summary:

Edible coatings act as barriers on the surface of fresh fruit and vegetables which maintain the quality, extend shelf-life and minimize microbial spoilage. Chitosan, salicylic acid (SA) and oxalic acid (OA) alone are used as edible coatings. The influence of chitosan emulsion, SA or OA alone and chitosan emulsion loaded with SA or OA on ethylene production, respiration rate and weight loss, fruit quality, chilling injury and disease incidence in late maturing 'Midknight Valencia' sweet orange fruit stored at 3ºC and 7°C for 56 and 84 days followed by 10 days simulated shelf conditions (21 ± 1 °C) was investigated. The chitosan emulsion (1.5%) loaded with 2.0 mM OA coating significantly suppressed mean ethylene production (5.73, 5.06 and 5.48 nmol kg^{-1} h^{-1}) compared to the control (9.38, 7.18 and 9.21 nmol Kg^{-1} h^{-1}) in the sweet orange during storage of fruit for 56 days cold storage followed by 10 days simulated shelf conditions, 84 days cold storage and 84 days cold storage followed by 10 days simulated shelf conditions. However, SA (2.0 mM) coating significantly suppressed mean respiration rate (0.63 mmol CO_2 $kg^{-1}h^{-1}$) compared to control (0.93 mmol CO_2 $kg^{-1}h^{-1}$) and all other treatments except the fruit coated with chitosan emulsion alone during cold storage of fruit for 56 days. The lowest respiration was observed at storage temperature (7°C) for all storage periods. Similarly, the chitosan (1.5%) emulsion resulted in higher fruit firmness (444.1 N), SSC:TA (16.15) than control and all other treatments. However, the fruit coated with chitosan (1.5%) loaded with 2.0 mM SA resulted in significantly ($P \leq 0.05$) higher SSC (12.30%), TA (0.75%), vitamin C (35.46 mg $100ml^{-1}$ FJ) and lowest level of weight loss (4.06%) compared to control and all other treatments for 84 days cold storage. When averaged over treatments, the temperature of 3°C resulted in a higher level of fruit firmness as compared to 7°C. Similarly, TA was higher at a temperature of 7°C at 56 days cold storage and 84 days cold storage compared to 3°C. The lowest disease incidence (6.2%) was noted in the fruit coated with 1.5% chitosan emulsion.

The treatment 2.0 mM OA gave lower chilling injury at 7°C for all storage periods. In conclusion, chitosan, SA and OA alone seem to be more effective than chitosan loaded with SA or OA, in reducing respiration rate and higher fruit firmness and total antioxidants during cold storage conditions in 'Midknight Valencia' sweet orange fruit.

8.1. Introduction

Sweet orange (*Citrus sinensis* L. Osbeck) is one of the major profitable fruit crops that is widely consumed both as fresh fruit and juice (Kalac and Krausová, 2005). Sweet orange fruit enjoy great popularity all over the world due to their good taste, higher vitamin C and antioxidants as well as widespread availability (Goristein et al., 2001; Liu et al., 2012). Citrus is grown in the world between 40˚ north and south latitude mainly in the tropical and subtropical areas (Ismail and Zhang, 2004). Domestic and international consumers prefer citrus fruit with high quality such as rind free from blemishes, symptoms of disease and pest damage; and glossy appearance with good taste (Hussain, 2014). After harvest, citrus fruits are susceptible to postharvest physiological disorders and microbiological decay. Generally, citrus fruits are characterized as non-climacteric fruit; hence they do not show the climacteric rise in ethylene production and respiration rate after harvest during fruit ripening, contrary to climacteric fruits like apple, plum, peach, pear and mango (Kader and Arpaia, 2002). Endogenous ethylene or exogenously applied ethylene may however still has an impact on fruit shelf life and quality of sweet oranges (Porat et al., 2000). Similarly, sweet orange fruit rate of respiration, which is an important determinant of the fruit shelf life, is influenced by temperature, humidity, movement of air, composition of gases, bruises and microbial infection (Murata, 1997). Preharvest factors affecting shelf life and quality include rootstock, cultivar, cultural practices, harvest conditions, and maturity stage, while the postharvest factors involve the operational efficiency, precooling, various treatments (eg. fungicide and waxes) to the fruit and storage conditions, as well as chilling injury (CI) during cold storage (Hatton, 1990; Paull, 1990; Kader and Arpaia, 2002). However, sweet orange cultivars may differ in severity and susceptibility to chilling injury. Several post-harvest treatments have been used to alleviate chilling sensitivity and decay of citrus fruit (Ben-Yehoshua et al., 1987, 1989; Wild, 1990), postharvest heat shock (Rab and Saltveit, 1996), anaerobic shock treatments (Pesis et al., 1994),

chemical treatments, packaging and waxing (Petracek et al., 1999). Application of fungicide(s) is used to control postharvest diseases but consumers are concerned about their potential injurious effects on health. Moreover, pathogens also develop resistance to repeated application of fungicides (Stefano et al., 2009; Ren and Shaoying, 2013). Therefore, new methods for controlling postharvest diseases which have good efficacy, low residues and little or no toxicity to non-target organisms are required.

Different kinds of edible coating materials are available on the market, mainly for intact fruits and vegetables, and research continues with the objective of developing better coatings that are capable of preserving, or even improving the quality of fruits and vegetables during storage. The advantages of various edible coatings in extending postharvest life and maintenance of fruit quality of a range of fruit crops have been described earlier in Chapter 2 and Chapter 4. Wax coating is commercially used to extend postharvest life of sweet orange in pack houses (Porat et al., 2005). Developing edible coatings for citrus fruits seem to be attractive alternatives over wax coating, because edible coatings are usually not injurious to human health and are environmentally friendly (Dhall, 2013).

Chitosan coating has been employed to prolong storage life and manage postharvest diseases of many fruits such as peach, citrus, strawberry, table grape, litchi, peach and plum fruit (Zhao et al., 2006). Chitosan can be combined with other compounds such as essential oils in order to enhance its antimicrobial activity (Perdones et al., 2012). Salicylic acid (SA) is a safe and natural compound found in plants which is used to reduce postharvest losses of horticultural commodities (Asghari and Aghdam, 2010). SA has been reported to maintain fruit firmness and reduce weight loss and postharvest diseases in different fruits, details of which have been described in the previous Chapters 2 and 4. The advantages of OA application in extending postharvest life and maintaining fruit quality, reducing postharvest diseases, chilling injury and other physiological disorders in climacteric and non-climacteric fruits have been previously detailed in Chapter 2 and Chapter 4.

The effects of different coating materials such as chitosan, SA and OA alone have so far been reported in this thesis for different fruits such as peach, citrus, strawberry, mango, sugar apple and litchi (Chapter 2 and 4). However the effects of

postharvest application of chitosan, and loaded with SA or OA and cold storage temperatures on the modulation of ethylene production, respiration rate and quality of sweet orange fruit are not known and warrants investigation. It was hypothesised that chitosan loaded with SA or OA will be more effective in reducing ethylene production, respiration rate and maintaining fruit quality in cold stored sweet orange fruit compared to the application of chitosan, SA or OA alone. Therefore the effects of chitosan, SA or OA alone and chitosan loaded with SA or OA on the modulation of ethylene production, respiration rate and weight loss, firmness, soluble solids concentrations (SSC), titratable acidity (TA), ratio between SSC:TA, vitamin C, total antioxidants, chilling injury and disease incidence in late maturing 'Midknight Valencia' sweet orange fruit stored at 3°C and 7°C for 56 and 84 days followed by 10 days to simulate shelf conditions (21 ± 1 °C) were investigated.

8.2. Materials and methods

8.2.1. Plant material

Sweet orange cv. Midknight Valencia fruits were harvested at commercial maturity based upon SSC and SSC/TA ratio, from Moora Citrus Orchard (30° 35' S/115° 55' E), Dandaragan, Western Australia. Uniform sized fruit, free from visible symptoms of diseases and blemishes were transported to the Horticulture Research Laboratory, Curtin University, Perth, WA, within four hours of harvest.

8.2.2. Treatments and experimental design

The experiment was conducted during the year 2014 - 2015. The ripe sweet orange fruit were coated with chitosan emulsion (1.5%), SA (2.0 mM) or OA (2.0 mM) alone or the chitosan emulsion (1.5%) loaded with SA (2.0 mM) or OA (2.0 mM). Tween 20 (0.25%) was used as a surfactant. Untreated fruit served as a control. Following the treatments, the fruit were kept at temperature (20 ± 1 °C) and relative humidity (60 ± 5%) for four hours to dry. After drying the fruit were divided into two groups and kept in cold storage (3°C and 7°C) and relative humidity (90 ± 5%). Ethylene production, respiration rate, fruit firmness, SSC, TA, SSC and TA ratio, levels of vitamin C total antioxidants, chilling injury and disease incidence were determined from the fruit stored at 3 ºC and 7 ºC for 56 and 84 days and followed by 10 days in simulated shelf conditions (21 ± 1 °C) for both storage periods. Fruit weight loss was recorded only at 56 and 84 days after cold storage. The experiment

was laid out by following two factors (treatments and storage temperatures) in a factorial completely randomized design. All the treatments were replicated four times and 20 fruit were included in each replication.

8.2.3. Determination of ethylene production

Ethylene production rate was determined by following the method of Pranamornkith et al. (2012) and Hussain (2014) as detailed in Chapter 3, Section 3.4 of this thesis. The ethylene production was determined by using an ETD 300 ethylene detector (Sensor sense B.V, Nijmegen, The Netherlands) and was expressed as nmol kg^{-1} h^{-1}.

8.2.4. Determination of rate of respiration

The rate of respiration was determined as carbon dioxide (CO_2) production from the fruit according to the method described by Zaharah (2011) which has also been described in Chapter 3, Section 3.5 of the thesis. All the estimations were performed twice and the rate of respiration was expressed as mmol CO_2 kg^{-1} h^{-1}.

8.2.5. Determination of percentage loss of fruit weight

Following each cold storage period, fruit weight loss was calculated as described in Chapter 3, Section 3.6. The weight loss was expressed as a percentage.

8.2.6. Determination of fruit firmness

The citrus fruit firmness was determined by using a fruit compression test. Five randomly selected fruit (75 mm high) were used for the fruit compression test using a textural analyser interfaced to a personal computer with Nexygen® software. The textural analyser was fitted with a 15 cm × 15 cm horizontal square base table. Each fruit was positioned between two flat plates with the stalk axis vertical to the plate. The crosshead speed was 200 mm min^{-1} and was used to compress fruit (50% of their height). The method has previously been explained in more detail in Chapter 3, Section 3.7.

8.2.7. Determination SSC, TA and SSC:TA ratio

The SSC was recorded by measuring the refractive index using an infrared digital refractometer (Atago-Palette PR 101, Atago CO. Ltd, Itabashi-Ku, Tokyo, Japan) as detailed in Chapter 3, Section 3.8. TA was determined by titrating the fresh fruit juice against 0.1 N NaOH and expressed as citric acid percentage. SSC: TA ratio was

calculated by dividing the SSC value with the corresponding TA value as described previously in Chapter 3, Section 3.8.

8.2.8. Determination of vitamin C

Vitamin C concentration in the freshly extracted juice from 10 sweet orange fruits was determined using a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, Dunmow, Essex, UK) according to Hussain (2014) and Pham (2009) with some modifications as outlined in Chapter 3, Section 3.10. Vitamin C concentration was calculated by using a standard curve of L-ascorbic acid and expressed as mg vitamin C per 100 ml fresh juice.

8.2.9. Determination of total antioxidants

The total antioxidants from freshly extracted juice from 10 sweet orange fruits were estimated by using the modified method of Hussain (2014) and Pham (2009). Total antioxidants was calculated using a standard curve of 6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (Trolox) and was expressed on a µM trolox equivalent antioxidant activity (TEAC) 100 ml^{-1} FJ basis. The detailed method has previously been explained in Chapter 3, Section 3.11.

8.2.10. Determination of chilling injury (CI)

The chilling injury was determined by ranking the fruit on a rating scale from 0 to 3 and expressed as chilling injury index as described earlier in Chapter 3, Section 3.12.

8.2.11. Determination of disease incidence

The disease incidence was determined by examining the fruit regularly. Fruit was regarded as infected if a visible lesion was observed and disease incidence was expressed as percentage. The detailed procedure has been explained in Chapter 3, Section 3.13.

8.2.12. Statistical analysis

The data were analysed by employing one-way or two-way analysis of variance (ANOVA), using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted experimental station, UK). The effects of coating treatments, storage temperature and their interactions on different parameters were evaluated within ANOVA. Least significant differences were calculated following significant F test at $P \leq 0.05$. To

ascertain the authenticity of statistical analysis, various assumptions of analysis were verified.

8.3. Results

8.3.1. Ethylene production

When averaged over different cold storage temperatures, all the treatments significantly ($P \leq 0.05$) suppressed mean ethylene production compared to the control in 'Midknight Valencia' sweet orange fruit following 56 day storage periods (Fig. 8.1). The fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) exhibited lower mean level of ethylene production (4.61 nmol kg^{-1} h^{-1}) compared to control (13.11 nmol kg^{-1} h^{-1}) and all other treatments at 56 days storage period but the differences were not significant. Meanwhile, the mean ethylene production was lowest (5.06 nmol kg^{-1} h^{-1}) in 84-day cold stored fruit which were coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) compared to control and all other treatments. However, the fruit coated with chitosan emulsion (1.5%) loaded with 2.0 mM OA suppressed mean ethylene production (5.73 and 5.48 nmol kg^{-1} h^{-1}) compared to controls (9.38 and 9.21 nmol kg^{-1} h^{-1}) and all other treatments in the fruit stored both for 56 and 84 days and simulated shelf conditions of 10 days respectively (Fig. 8.1). When averaged over different treatments, the mean ethylene production significantly ($P \leq 0.05$) increased in the fruit stored at 7°C compared to those stored at 3°C for 56 and 84 days and following 10 days simulated shelf condition. The interactions between different cold storage temperatures and treatments were found to be significant for ethylene production in 'Midknight Valencia' sweet orange fruit at all storage and simulated shelf condition periods.

8.3.2. Respiration rate

When averaged over different cold storage temperatures, the fruit coated with SA (2.0 mM) exhibited significantly ($P \leq 0.05$) lower respiration rate (0.63 mmol CO_2 kg^{-1} h^{-1}) compared to the control (0.93 mmol CO_2 kg^{-1} h^{-1}) and all other treatments except the fruit coated with 1.5% chitosan emulsion alone in fruit stored for 56 days (Table 8.1). When averaged over both cold storage temperatures, none of the treatments significantly affected the mean respiration rate in 84 days stored fruit. Fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) suppressed mean respiration rate (0.44 mmol CO_2 kg^{-1} h^{-1}) compared to the control fruit (0.64

mmol CO_2 kg^{-1} h^{-1}) and all other treatments following 84 days cold storage and simulated shelf conditions. When averaged over treatments, mean respiration rate was significantly higher when stored at 3 °C (0.95 mmol CO_2 kg^{-1} h^{-1}) than the fruit stored at 7 °C (0.62 mmol CO_2 kg^{-1} h^{-1}) only in 56 days stored fruit followed by 10-day simulated shelf conditions. The interactions between the treatments and different cold storage temperatures were found to be significant for respiration rate at all storage periods and simulated shelf conditions except 84 days storage followed by 10-day simulated shelf conditions (Table 8.1).

8.3.3. Weight loss

All the treatments except chitosan alone and chitosan loaded with SA have significantly reduced mean weight loss when averaged over storage temperatures, as compared to control in 56 days stored fruit (Table 8.2). Whilst, in 84 days stored fruit, all the treatments have significantly reduced mean fruit weight loss as compared to the control. When averaged over different cold storage temperatures, the mean fruit weight loss was higher in the fruit stored at 7 °C than those kept at 3 °C for 56 days and 84 days. The interaction between the treatments and different cold storage temperatures was found to be significant for weight loss in fruit stored for 56 days only but not significant for 84 days stored fruit (Table 8.2).

8.3.4. Firmness

'Midknight Valencia' sweet orange fruit coated with chitosan emulsion (1.5%) alone exhibited significantly higher mean firmness (444.10 N) as compared with control (362.0 N) and all other treatments except the fruit coated with 2.0 mM OA alone at 56 days of cold storage period (Table 8.3). Meanwhile, the fruit coated with chitosan emulsion (1.5%) alone showed significantly highest mean fruit firmness (425.8 N) as compared to control and all other treatments following 56 days cold storage and 10-day simulated shelf conditions. When averaged over different cold storage temperatures, significantly highest mean fruit firmness (399.0 N and 374.9 N) was observed in the fruit coated with chitosan emulsion (1.5%) alone compared to controls (319.1 N and 259.1 N) and all other treatments in both 84 days cold storage as well as 84 days cold storage and 10-day simulated shelf conditions respectively. When averaged over treatments, mean firmness was significantly lower in the fruit stored at 7 °C than 3 °C irrespective of storage periods. The interactions between the

treatments and different cold storage temperatures were not significant for fruit firmness under all storage periods and simulated shelf conditions.

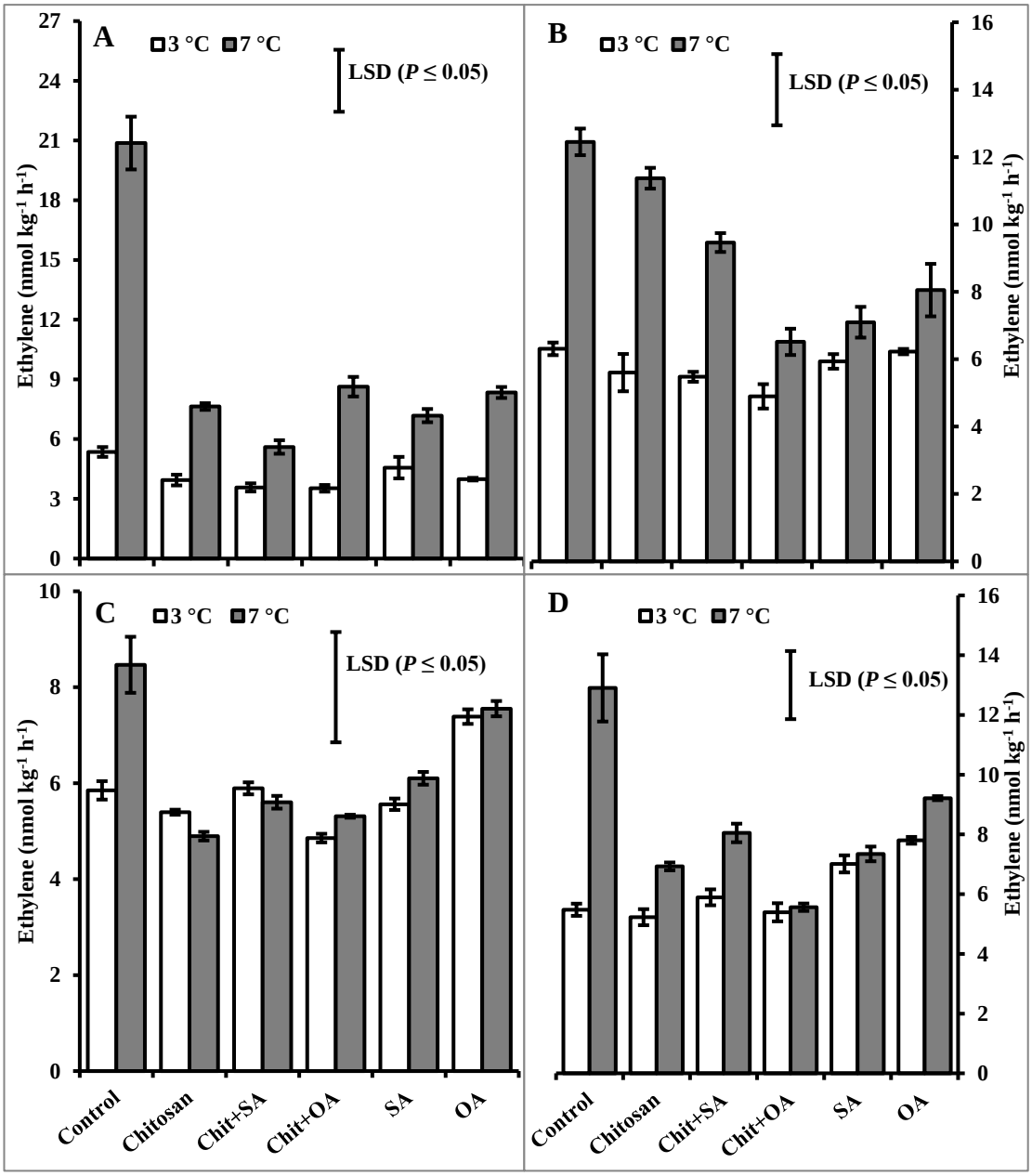


Figure 8.1. A–D. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on ethylene production at (A) 56 days cold (3°C and 7°C) storage, (B) 56 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions, (C) 84 days cold (3°C and 7°C) storage and (D) 84 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions in 'Midknight Valencia' sweet orange fruit. Vertical bars represent SE, n = four replicates, two fruit per replication.

Table 8.1. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on rate of respiration at 56 days cold (3°C and 7°C) storage, 56 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions, 84 days cold (3°C and 7°C) storage and 84 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions in 'Midknight Valencia' sweet orange fruit.

Respiration rate (mmol CO_2 kg^{-1} h^{-1})				
Treatments	3 °C	7 °C	Means (T)	LSD ($P \leq 0.05$)
56 days cold storage				Treatment(T)= 0.12, Temperature(Tem)= NS, T X Tem= 0.17
Control	0.84	1.03	0.93 a	
Chitosan	0.71	0.71	0.71 bc	
Chitosan + salicylic acid	0.86	0.68	0.77 b	
Chitosan + oxalic acid	0.94	0.72	0.83 ab	
Salicylic acid	0.63	0.63	0.63 c	
Oxalic acid	0.76	0.80	0.78 b	
Means (Tem)	0.79	0.76		
56 cold storage followed by 10 days simulated shelf conditions				Treatment(T)= NS, Temperature(Tem)= 0.08, T X Tem= 0.20
Control	0.83	0.79	0.81	
Chitosan	1.14	0.52	0.83	
Chitosan + salicylic acid	1.07	0.59	0.83	
Chitosan + oxalic acid	1.02	0.52	0.77	
Salicylic acid	0.82	0.72	0.77	
Oxalic acid	0.85	0.55	0.70	
Means (Tem)	0.95a	0.62b		
84 days cold storage				Treatment(T)= NS, Temperature(Tem)= NS, T X Tem= 0.17
Control	0.90	0.71	0.80	
Chitosan	0.79	0.91	0.85	
Chitosan + salicylic acid	1.04	0.83	0.93	
Chitosan + oxalic acid	0.88	0.69	0.79	
Salicylic acid	0.69	0.86	0.78	
Oxalic acid	0.91	0.82	0.86	
Means (Tem)	0.87	0.80		
84 cold storage followed by 10 days simulated shelf conditions				Treatment(T)= 0.11, Temperature(Tem)= NS, T X Tem= NS
Control	0.61	0.68	0.64a	
Chitosan	0.53	0.57	0.55ab	
Chitosan + salicylic acid	0.47	0.41	0.44b	
Chitosan + oxalic acid	0.55	0.56	0.55ab	
Salicylic acid	0.52	0.43	0.47b	
Oxalic acid	0.56	0.40	0.48b	
Means (Tem)	0.54	0.51		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (two fruit per replication).

Table 8.2. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid or 2.0 mM oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid on weight loss at 56 and 84 days cold (3°C and 7°C) storage in 'Midknight Valencia' sweet orange fruit.

Weight loss (%) 56 days				
Treatment	3 °C	7 °C	Mean (T)	LSD ($P \leq 0.05$)
Control	2.54	5.38	3.96a	Treatments (T) = 0.71,
Chitosan	2.25	5.09	3.67ab	Temperature (Tem) =
Chitosan + salicylic acid	1.93	3.98	2.95bc	0.41, T x Tem = 1.01
Chitosan + oxalic acid	2.12	4.45	3.28abc	
Salicylic acid	2.43	3.32	2.87c	
Oxalic acid	2.07	3.07	2.57c	
Means (Tem)	2.22 b	4.21 a		
Weight loss (%) 84 days				
Control	9.71	12.1	10.9a	Treatments (T) = 0.65,
Chitosan	2.83	5.64	4.24d	Temperature (Tem) =
Chitosan + salicylic acid	2.71	5.40	4.06d	0.37, T x Tem = NS
Chitosan + oxalic acid	3.33	5.73	4.53d	
Salicylic acid	5.81	7.49	6.65c	
Oxalic acid	6.96	8.99	7.97b	
Means (Tem)	5.23 b	7.56 a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (20 fruit per replication).

Table 8.3. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on firmness at 56 days cold (3°C and 7°C) storage, 56 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions, 84 days cold (3°C and 7°C) storage and 84 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions in 'Midknight Valencia' sweet orange fruit.

Firmness (N)				
Treatments	3 °C	7 °C	Means (T)	LSD ($P \leq 0.05$)
56 days cold storage				Treatment (T)= 17.27, Temperature (Tem)= 9.97, T X Tem= NS
Control	387.8	336.3	362.0 c	
Chitosan	449.4	438.7	444.1 a	
Chitosan + salicylic acid	424.4	403.1	413.8 b	
Chitosan + oxalic acid	412.0	396.5	404.3 b	
Salicylic acid	416.1	399.1	407.6 b	
Oxalic acid	435.1	432.4	433.8 a	
Means (Tem)	420.8 a	401.0 b		
56 days cold storage followed by 10 days simulated shelf conditions				Treatment (T)= 15.73, Temperature (Tem)= 9.08, T X Tem= NS
Control	350.0	323.3	336.6 d	
Chitosan	440.6	410.9	425.8 a	
Chitosan + salicylic acid	381.0	369.9	375.5 c	
Chitosan + oxalic acid	402.9	389.2	396.0 b	
Salicylic acid	365.6	360.5	363.0 c	
Oxalic acid	388.0	370.2	379.1 c	
Means (Tem)	388.0a	370b		
84 days cold storage				Treatment (T)= 7.43, Temperature (Tem)= 4.29, T X Tem= NS
Control	320.3	317.8	319.1 e	
Chitosan	403.7	394.3	399.0 a	
Chitosan + salicylic acid	365.6	355.3	360.4 bc	
Chitosan + oxalic acid	366.6	363.4	365.0 b	
Salicylic acid	351.1	339.4	345.3 d	
Oxalic acid	359.4	346.6	353.0 c	
Means (Tem)	361.1a	352.8b		
84 days cold storage followed by 10 days simulated shelf conditions				Treatment (T)= 13.56, Temperature (Tem)= 7.83, T X Tem= NS
Control	266.5	251.8	259.1 e	
Chitosan	382.0	367.8	374.9 a	
Chitosan + salicylic acid	321.4	306.8	314.1 bc	
Chitosan + oxalic acid	331.1	323.3	327.2 b	
Salicylic acid	308.9	285.6	297.2 d	
Oxalic acid	312.2	289.5	300.8 cd	
Means (Tem)	320.3a	304.1b		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

8.3.5. Soluble solids concentration (SSC)

When averaged over storage temperatures, the fruit coated with chitosan emulsion (1.5%) alone exhibited significantly ($P \leq 0.05$) higher mean SSC (11.84%) compared to the control (10.81%) and all other treatments except the fruit coated with 2.0 mM SA alone at 56 days of storage (Table 8.4). Whilst, the fruit stored for 56 days followed by 10-day simulated shelf conditions which were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) exhibited significantly ($P \leq 0.05$) higher mean SSC (11.80%) compared to the control (10.61%) and all other treatments except the fruit coated with 1.5% chitosan emulsion alone and 2.0 mM OA alone. Similarly, when averaged over storage temperatures, fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) resulted in the significantly ($P \leq 0.05$) highest mean SSC (12.30%) compared to the control (11.10%) and all other treatments at 84 days of cold storage. Meanwhile, the fruit coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) showed significantly ($P \leq 0.05$) highest mean SSC (11.67%) compared to the control (10.89%) and all other treatments at 84 days cold storage followed by 10-day simulated shelf conditions. When averaged over different treatments, mean SSC was significantly higher in the fruit stored at 3 °C (11.50% and 11.53%) than 7 °C (11.16% and 11.16%) at 56 days cold storage and 56 days storage followed by 10-day simulated shelf conditions respectively. Whilst, mean SSC when averaged over treatments was significantly higher in the fruit stored at 7 °C (11.85% and 11.53%) than at 3 °C (11.48% and 11.14%) at 84 days and 84 days followed by 10-day simulated shelf conditions respectively. The interactions between the treatments and different temperatures were found to be significant for SSC at all storage periods except at 84 days cold storage (Table 8.4).

8.3.6. Titratable acidity (TA)

When averaged over both storage temperatures, all the treatments did not significantly affect TA in ‘Midknight Valencia fruit stored for 56, 84 days and stored for 84 days followed by 10-day simulated shelf conditions (Table 8.5). When averaged over different treatments, the mean level of TA did not differ significantly in the fruit stored at 7 °C than 3 °C storage during all storage periods followed by 10 days simulated shelf conditions except at 56 days cold storage. The interactions between different treatments and storage temperatures for acidity differed

significantly only in 84-days cold stored fruit followed by 10 days simulated shelf conditions in 'Midknight Valencia' sweet orange (Table 8.5).

8.3.7. SSC:TA ratio

All the treatments except OA (2.0 mM) significantly increased mean SSC:TA ratio as compared to the control after 56 days of cold storage (Table 8.6). Meanwhile, SA (2.0 mM) coated fruit showed highest mean SSC:TA ratio (17.46) compared to control and all other treatments at 56 days cold storage fruit followed by 10-day simulated shelf conditions (Table 8.6). At 84 days of cold storage, the mean level of SSC:TA ratio was not affected significantly by any of the treatments as compared to the control. When averaged over cold storage temperatures, 'Midknight Valencia' fruit coated with OA (2.0 mM) exhibited highest SSC:TA ratio (15.86) compared to control (14.64) and all other treatments at 84 days cold storage fruit followed by 10-day simulated shelf conditions. When averaged over both cold storage temperatures, the mean SSC:TA ratio was significantly higher (15.67) in the 'Midknight Valencia' fruit stored at 3°C than those stored at 7°C (14.56) for 56 days and the trend was reversed in 84 days cold storage fruit followed by 10-day simulated shelf conditions . The interactions between different treatments and cold storage temperatures were found to be significant ($P \leq 0.05$) for SSC:TA only for 84 days cold storage and 84 days cold storage fruit followed by 10-day simulated shelf conditions.

Table 8.4. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on SSC at 56 days cold (3°C and 7°C) storage, 56 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions, 84 days cold (3°C and 7°C) storage and 84 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions in 'Midknight Valencia' sweet orange fruit.

SSC (%)				
Treatments	3 °C	7 °C	Means (T)	LSD ($P \leq 0.05$)
56 days cold storage				Treatment(T) = 0.29, Temperature(Tem)= 0.17, T X Tem= 0.42
Control	11.05	10.58	10.81c	
Chitosan	12.13	11.55	11.84a	
Chitosan + salicylic acid	11.78	11.03	11.40b	
Chitosan + oxalic acid	11.45	11.13	11.29b	
Salicylic acid	11.88	11.65	11.76a	
Oxalic acid	10.75	11.03	10.89c	
Means (Tem)	11.50a	11.16b		
56 days cold storage followed by 10 days simulated shelf conditions				Treatment(T)= 0.39, Temperature(Tem)= 0.23, T X Tem= 0.55
Control	10.75	10.48	10.61d	
Chitosan	12.00	11.08	11.54ab	
Chitosan + salicylic acid	12.20	11.40	11.80a	
Chitosan + oxalic acid	11.35	10.70	11.03c	
Salicylic acid	11.18	11.58	11.38bc	
Oxalic acid	11.73	11.75	11.74ab	
Means (Tem)	11.53a	11.16b		
84 days cold storage				Treatment(T)= 0.43, Temperature(Tem)= 0.25, T X Tem= NS
Control	10.95	11.25	11.10c	
Chitosan	11.55	11.75	11.65b	
Chitosan + salicylic acid	12.08	12.53	12.30a	
Chitosan + oxalic acid	11.28	11.68	11.47bc	
Salicylic acid	11.53	11.85	11.69b	
Oxalic acid	11.53	12.05	11.79b	
Means (Tem)	11.48b	11.85a		
84 days cold storage followed by 10 days simulated shelf conditions				Treatment(T)= 0.13, Temperature(Tem)= 0.08, T X Tem= 0.19
Control	10.85	10.93	10.89c	
Chitosan	11.60	11.13	11.36b	
Chitosan + salicylic acid	11.08	11.73	11.40b	
Chitosan + oxalic acid	11.25	11.55	11.40b	
Salicylic acid	11.18	11.40	11.29b	
Oxalic acid	10.90	12.45	11.67a	
Means (Tem)	11.14b	11.53a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 8.5. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on TA at 56 days cold (3°C and 7°C) storage, 56 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions, 84 days cold (3°C and 7°C) storage and 84 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions in 'Midknight Valencia' sweet orange fruit.

Titratable acidity (%) 56 days cold storage				
Treatments	3 °C	7 °C	Means (T)	LSD ($P \leq 0.05$)
Control	0.778	0.816	0.797	Treatment (T) = NS,
Chitosan	0.730	0.739	0.734	Temperature (Temp)=
Chitosan + salicylic acid	0.749	0.730	0.739	0.027, T x Temp = NS
Chitosan + oxalic acid	0.720	0.749	0.734	
Salicylic acid	0.710	0.787	0.749	
Oxalic acid	0.739	0.797	0.768	
Means (Temp)	0.74b	0.77a		
56 days cold storage followed by 10 days simulated shelf conditions				
Control	0.730	0.749	0.739a	Treatment (T) = 0.056,
Chitosan	0.691	0.710	0.701abc	Temperature (Temp)=
Chitosan + salicylic acid	0.778	0.710	0.744a	NS, T x Temp = NS
Chitosan + oxalic acid	0.672	0.662	0.667bc	
Salicylic acid	0.662	0.653	0.658c	
Oxalic acid	0.682	0.758	0.72ab	
Means (Temp)	0.702	0.707		
84 days cold storage				
Control	0.682	0.758	0.72	Treatment (T) = NS,
Chitosan	0.634	0.730	0.682	Temperature (Temp)=
Chitosan + salicylic acid	0.749	0.749	0.749	NS, T x Temp = NS
Chitosan + oxalic acid	0.701	0.691	0.696	
Salicylic acid	0.701	0.672	0.686	
Oxalic acid	0.730	0.710	0.72	
Means (Temp)	0.699	0.718		
84 days cold storage followed by 10 days simulated shelf conditions				
Control	0.730	0.758	0.744	Treatment (T) = NS,
Chitosan	0.778	0.672	0.725	Temperature (Temp)=
Chitosan + salicylic acid	0.739	0.768	0.754	NS, T x Temp = 0.033
Chitosan + oxalic acid	0.700	0.778	0.739	
Salicylic acid	0.750	0.730	0.744	
Oxalic acid	0.768	0.71d	0.739	
Means (Temp)	0.746	0.736		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 8.6. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on SSC/TA ratio at 56 days cold (3°C and 7°C) storage, 56 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions, 84 days cold (3°C and 7°C) storage and 84 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions in 'Midknight Valencia' sweet orange fruit.

SSC / TA ratio 56 days cold storage				
Treatments	3 °C	7 °C	Means (T)	LSD ($P \leq 0.05$)
Control	14.41	12.98	13.70b	Treatment (T) = 0.96,
Chitosan	16.65	15.66	16.15a	Temperature (Temp)=
Chitosan + salicylic acid	15.73	15.17	15.45a	0.55, T x Temp = NS
Chitosan + oxalic acid	15.94	14.87	15.41a	
Salicylic acid	16.75	14.81	15.78a	
Oxalic acid	14.55	13.85	14.20b	
Means (Temp)	15.67a	14.56b		
56 days cold storage followed by 10 days simulated shelf conditions				
Control	14.86	14.02	14.44c	Treatment (T) = 1.25,
Chitosan	17.41	15.69	16.55ab	Temperature (Temp)=
Chitosan + salicylic acid	15.71	16.06	15.88b	NS, T x Temp = NS
Chitosan + oxalic acid	17.00	16.19	16.60ab	
Salicylic acid	16.95	17.97	17.46a	
Oxalic acid	17.31	15.50	16.41ab	
Means (Temp)	16.54	15.90		
84 days cold storage				
Control	16.19	14.90	16.19	Treatment (T) = NS,
Chitosan	18.38	16.21	18.38	Temperature (Temp)=
Chitosan + salicylic acid	16.14	16.73	16.14	NS, T x Temp = 1.74
Chitosan + oxalic acid	16.13	17.02	16.13	
Salicylic acid	16.46	17.65	16.46	
Oxalic acid	15.81	17.03	15.81	
Means (Temp)	16.52	16.59		
84 days cold storage followed by 10 days simulated shelf conditions				
Control	14.87	14.41	14.64c	Treatment (T) = 0.56,
Chitosan	14.99	16.57	15.78a	Temperature (Temp)=
Chitosan + salicylic acid	14.99	15.27	15.13bc	0.32, T x Temp = 0.79
Chitosan + oxalic acid	16.08	14.86	15.47ab	
Salicylic acid	14.74	15.62	15.18bc	
Oxalic acid	14.19	17.54	15.86a	
Means (Temp)	14.98b	15.71a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

8.3.8. Vitamin C

When averaged over cold storage temperatures, the mean levels of vitamin C were not significantly affected by different treatments as compared to the control at any storage conditions except at 84 days cold storage followed by 10-day simulated shelf conditions (Table 8.7). When averaged over different treatments, the mean levels of vitamin C were also not significantly affected by both cold storage temperatures after 56 days cold storage as well as 56 days cold storage followed by 10-day simulated shelf conditions (Table 8.7). Meanwhile, after 84 days cold storage and 84 days cold storage followed by 10-day simulated shelf conditions, fruit coated with OA (2.0 mM) exhibited the significantly highest mean level of vitamin C (31.13 mg $100ml^{-1}$ FJ) as compared to the control and all other treatments. When averaged over different treatments, the mean levels of vitamin C were significantly higher (35.55 mg $100ml^{-1}$ FJ and 31.89 mg $100ml^{-1}$ FJ) in the fruit stored at 7 ºC than those stored at 3 ºC for 84 days and 84 days storage followed by 10-day simulated shelf conditions (Table 8.7). The interaction between different treatments and cold storage temperatures for levels of vitamin C was only significant for 84 days stored fruit followed by 10-day simulated shelf conditions.

8.3.9. Total antioxidants

When averaged over different cold storage temperatures, all treatments except OA (2.0 mM) alone reduced the mean total antioxidants as compared to the control in 56 and 84 days cold storage as well as 56 days cold storage fruit followed by 10-day simulated shelf conditions, but the differences were not significant (Table 8.8). Meanwhile, 84 days cold stored fruit followed by 10-day simulated shelf conditions, which were previously coated with 2.0 mM OA showed significantly highest mean level of antioxidants (47.21 µM Trolox $100ml^{-1}$ FJ) as compared to the control and all other treatments. When averaged over treatments, mean total antioxidant levels were higher when fruit was stored at 3°C compared to 7°C for periods of 56 and 84 days. Meanwhile, when averaged over all treatments, mean total antioxidant level was significantly ($P \leq 0.05$) higher when fruit was stored at 7°C compared to 3°C for 56 and 84 days storage followed by 10-day simulated shelf conditions. The interactions between different treatments and storage temperatures were found to be significant ($P \leq 0.05$) for total antioxidants in ‘Midknight Valencia’ oranges stored

for 56 days cold storage, 56 days cold storage followed by 10-day simulated shelf conditions and 84 days cold storage followed by 10-day simulated shelf conditions.

8.3.10. Chilling injury (CI)

As expected, there was no chilling injury on control and treated 'Midknight Valencia' sweet orange fruit when stored at 7°C for 56 days followed by 10-day simulated shelf conditions (Table 8.9). However, when averaged over both cold storage temperatures, the 'Midknight Valencia' sweet orange fruit coated with 2.0 mM OA exhibited lowest mean CI (0) compared to control (0.29) and all other treatments at 84 days storage followed by 10-day simulated shelf conditions (Table 8.10). The interaction between treatments and different cold storage temperatures on level of CI was found to be not significant only in 84 days stored fruit followed by 10-day simulated shelf conditions.

8.3.11. Disease incidence

There was no disease incidence noted on 'Midknight Valencia' sweet orange fruit stored at 3 °C or 7 °C during 56 days cold storage as well as 56 days cold storage followed by 10-day simulated shelf conditions. When the fruit were kept at both cold storage temperatures for 84 days followed by 10-day simulated shelf conditions, no disease was recorded on the fruit stored at 3 °C. All the treatments exhibited disease incidence as compared to the control for fruit stored at 7 °C for 84 days and followed by 10-day simulated shelf conditions. The fruit coated with chitosan emulsion (1.5%) alone exhibited lowest disease incidence (6.2%) compared to the control fruit (22.5%) and all other treatments following 84 days cold storage and 10-day simulated shelf conditions (Fig. 8.2).

Table 8.7. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on vitamin C at 56 days cold (3°C and 7°C) storage, 56 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions, 84 days cold (3°C and 7°C) storage and 84 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions in 'Midknight Valencia' sweet orange fruit.

Vitamin C (mg $100ml^{-1}$ FJ) at 56 days cold storage				
Treatments	3 °C	7 °C	Means (T)	LSD ($P \leq 0.05$)
Control	53.90	41.00	47.40	Treatment (T) = NS,
Chitosan	38.40	39.50	39.00	Temperature (Temp)=
Chitosan + salicylic acid	42.50	36.40	39.40	NS, T x Temp = NS
Chitosan + oxalic acid	38.10	40.50	39.30	
Salicylic acid	38.10	43.00	40.60	
Oxalic acid	36.90	41.20	39.00	
Means (Temp)	41.3	40.3		
56 days cold storage followed by 10 days simulated shelf conditions				
Control	40.97	43.20	42.08	Treatment (T) = NS,
Chitosan	44.14	45.82	44.98	Temperature (Temp)=
Chitosan + salicylic acid	45.47	39.09	42.28	NS, T x Temp = NS
Chitosan + oxalic acid	39.90	48.48	44.19	
Salicylic acid	41.81	39.90	40.85	
Oxalic acid	40.93	42.46	41.69	
Means (Temp)	42.20	43.16		
84 days cold storage				
Control	33.65	36.76	35.20	Treatment (T) = NS,
Chitosan	30.90	32.84	31.87	Temperature (Temp)=
Chitosan + salicylic acid	33.04	37.89	35.46	1.91, T x Temp = NS
Chitosan + oxalic acid	30.77	34.88	32.82	
Salicylic acid	29.80	34.62	32.21	
Oxalic acid	32.74	36.34	34.54	
Means (Temp)	31.82b	35.55a		
84 days cold storage followed by 10 days simulated shelf conditions				
Control	28.73	31.68	30.20 b	Treatment (T) = 0.74,
Chitosan	27.24	30.70	28.97 c	Temperature (Temp)=
Chitosan + salicylic acid	26.66	32.03	29.34 c	0.43, T x Temp = 1.05
Chitosan + oxalic acid	27.89	32.55	30.22 b	
Salicylic acid	27.50	30.09	28.79 c	
Oxalic acid	27.95	34.30	31.13 a	
Means (Temp)	27.66b	31.89a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 8.8. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on total antioxidants at 56 days cold (3°C and 7°C) storage, 56 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions, 84 days cold (3°C and 7°C) storage and 84 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions in 'Midknight Valencia' sweet orange fruit.

Total antioxidants (µM Trolox 100 ml^{-1} FJ) 56 days cold storage				
Treatments	3 °C	7 °C	Means (T)	LSD ($P \leq 0.05$)
Control	46.09	45.66	45.88 ab	Treatment (T) = 0.65,
Chitosan	43.52	42.41	42.97 c	Temperature (Temp)=
Chitosan + salicylic acid	41.04	40.96	41.00 d	NS, T x Temp = 0.93
Chitosan + oxalic acid	46.16	44.40	45.28 b	
Salicylic acid	42.83	43.38	43.10 c	
Oxalic acid	45.66	46.58	46.12 a	
Means (Temp)	44.22	43.90		
56 days cold storage followed by 10 days simulated shelf conditions				
Control	46.53	47.38	46.96 a	Treatment (T) = 0.80,
Chitosan	43.65	43.65	43.65 c	Temperature (Temp)=
Chitosan + salicylic acid	42.31	42.51	42.41 d	0.46, T x Temp = 1.13
Chitosan + oxalic acid	45.12	44.94	45.03 b	
Salicylic acid	43.10	45.39	44.25 bc	
Oxalic acid	46.63	47.53	47.08 a	
Means (Temp)	44.56b	45.23a		
84 days cold storage				
Control	47.11	45.68	46.40 a	Treatment (T) = 0.60,
Chitosan	43.47	42.80	43.14 d	Temperature (Temp)=
Chitosan + salicylic acid	42.48	41.96	42.22 e	0.35, T x Temp = NS
Chitosan + oxalic acid	45.17	44.79	44.98 b	
Salicylic acid	44.15	44.35	44.25 c	
Oxalic acid	47.31	46.41	46.86 a	
Means (Temp)	44.95a	44.33b		
84 days cold storage followed by 10 days simulated shelf conditions				
Control	45.79	46.98	46.39 bc	Treatment (T) = 0.56,
Chitosan	45.99	45.64	45.81 c	Temperature (Temp)=
Chitosan + salicylic acid	43.80	45.76	44.78 d	0.32, T x Temp = 0.80
Chitosan + oxalic acid	45.76	45.96	45.86 c	
Salicylic acid	46.21	46.73	46.47 b	
Oxalic acid	46.56	47.85	47.21 a	
Means (Temp)	45.68b	46.49a		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 8.9. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on chilling injury index in 'Midknight Valencia' sweet orange fruit at 56 days cold (3°C) storage followed by 10 days simulated shelf conditions.

Chilling injury index at 56 days cold storage followed by 10 days simulated shelf conditions		
Treatments	3 °C	LSD ($P \leq 0.05$)
Control	0.25	Treatment (T) = NS
Chitosan	0.05	
Chitosan + salicylic acid	0.15	
Chitosan + oxalic acid	0.08	
Salicylic acid	0.13	
Oxalic acid	0.05	
Means (Temp)	0.116	

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

Table 8.10. Effects of 1.5% chitosan emulsion, 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on chilling injury index in 'Midknight Valencia' sweet orange fruit at 84 days cold (3°C and 7°C) storage followed by 10 days simulated shelf conditions.

Chilling injury index at 84 days cold storage followed by 10 days simulated shelf conditions				
Treatments	3 °C	7 °C	Means (T)	LSD ($P \leq 0.05$)
Control	0.325	0.25	0.287a	Treatment (T) = 0.11,
Chitosan	0.10	0.05	0.075b	Temperature (Temp)=
Chitosan + salicylic acid	0.20	0.175	0.187a	NS, T x Temp = NS
Chitosan + oxalic acid	0.05	0.05	0.05 b	
Salicylic acid	0.025	0.00	0.012b	
Oxalic acid	0.00	0.00	0.00 b	
Means (Temp)	0.117	0.087		

Any two means within a column and a row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (ten fruit per replication).

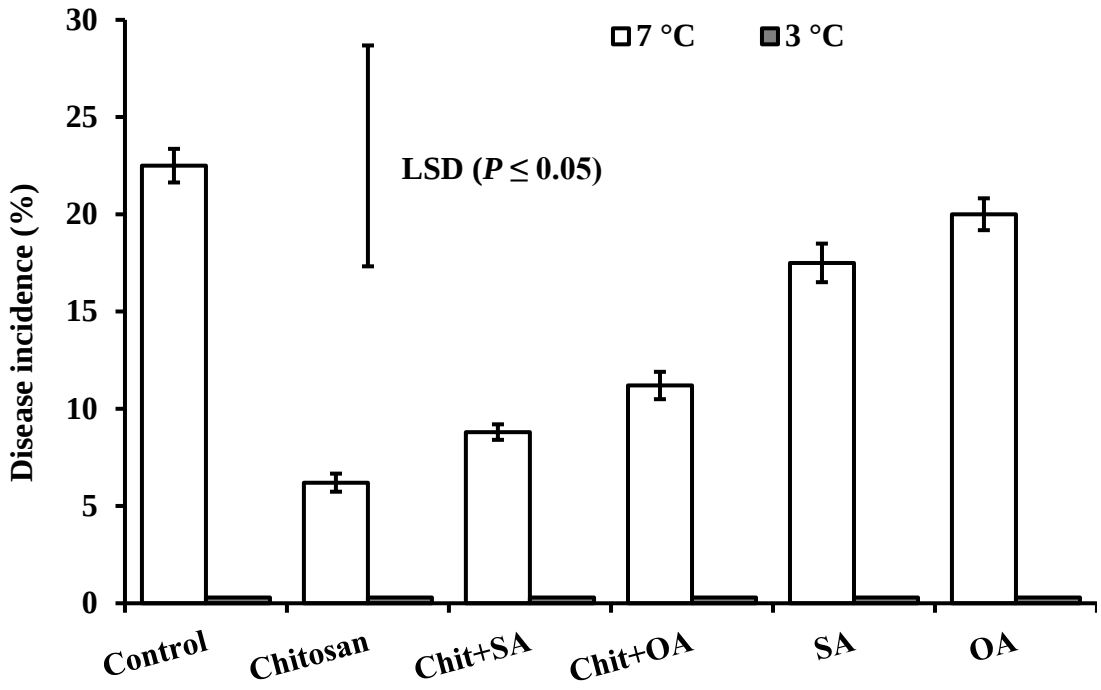


Figure 8.2. Effects of 1.5% chitosan emulsion (Chit), 2.0 mM salicylic acid (SA) or 2.0 mM oxalic acid (OA) alone and chitosan emulsion loaded with SA or OA on disease incidence after 84 days cold storage at 3°C and 7°C for 10-day simulated shelf conditions in 'Midknight Valencia' sweet orange fruit. Vertical bars represent SE, n = four replicates, ten fruit per replication.

8.4. Discussion

Edible coatings are known to modify gaseous composition around fresh horticultural produce, reduce loss of moisture and postharvest decay, maintain appearance, extend shelf life and maintain fruit quality with varying levels of success during postharvest handling phase (Baldwin et al., 1995; Petersen et al., 1999; Romanazzi et al., 2003; Cha and Chinnan, 2004; Valverde et al., 2005). Edible coating materials such as alginate, cellulose, chitosan, chitin, lipids, mucilage, milk protein, starch, wax, and zein are used and create an atmosphere similar to modified atmosphere packaging (MAP) and also show intrinsic biocide activity (Cha and Chinnan, 2004). Better maintenance of fruit quality was observed by using chitosan on peach (Li and Yu, 2001), nectarine (Giacalone and Chiabrando, 2015), strawberry (Vu et al., 2011) and papaya (Asgar et al., 2011). Some studies reported that SA has improved storability, prolonged shelf life and lowered fruit decay in peach (Khademi and Ershadi, 2013) and plum (Davarynjad et al., 2013). OA application has been reported to delay fruit

ripening, decrease ethylene production, maintain fruit quality and disease resistance in various fruits such as peach (Zheng et al., 2007a), mango (Zheng et al., 2007b) and plum (Wu et al., 2011). There are ample reports on the effects of wax coating, and other coatings such as cellulose, proteins and lipids on extending storage life and maintaining fruit quality of citrus fruits postharvest (Ladaniya, 2007). However, there is no information available on the effects of postharvest application of chitosan emulsion loaded with SA or OA on ethylene production, respiration rate and fruit quality of sweet orange cultivar 'Midknight Valencia' stored at 3 ºC and 7 ºC followed by simulated shelf conditions. The results obtained from this study have been discussed in light of the previous observations by other researchers.

8.4.1. Ethylene production

All the fruit of 'Midknight Valencia' coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) or SA (2.0 mM) suppressed the climacteric ethylene production compared to the control and all other treatments at all cold storage periods (Fig. 8.1). Edible coatings such as chitosan are considered to be good barriers on the surface of fresh fruit and vegetables. Presence of O_2 plays an important role in the ethylene biosynthesis (Abeles et al., 1992). When fruit are coated with chitosan it acts as a protective barrier which prevents the entry of oxygen into the fruit which ultimately reduces the level of endogenous ethylene (Noh, 2005). The effect of edible coating has been previously reported by different researchers that chitosan coatings can delay the ripening of tomatoes (El Ghaouth et al., 1992b). The beneficial effects of OA applications in inhibiting ethylene biosynthesis have been previously reported on some fruits such as plum (Wu et al., 2011), mango (Zhing et al., 2007b) and Chinese jujube (Wang et al., 2009). In addition, Mo et al., (2008) reported that SA treatment inhibited ethylene production on sugar apple. SA application has also been reported to retard the production of ethylene in plum (Lue et al., 2011) and strawberry (Babalar et al., 2007). Similarly the experimental results of this thesis show that 'Midknight Valencia' sweet orange fruit coated with chitosan (1.5%) loaded with OA resulted in lower rates of ethylene production compared to control fruit during the different periods of storage.

8.4.2. Respiration

The CO_2 production which was used as an indication of respiration rate was significantly decreased for 'Midknight Valencia' when fruit were coated with 2.0 mM SA than 2.0 mM OA, 1.5% chitosan emulsion alone and chitosan loaded with SA as well as OA (Table 8.1). It has been previously reported that edible coatings act as a protective barrier on the fruit surface which reduces availability of oxygen and ultimately reduces the respiration rate and also delays the ripening of fruit (Du et al., 1997; El Ghaouth et al., 1991; Jiang and Li, 2001).

8.4.3. Weight loss

When averaged over temperature, the lowest mean weight loss (2.57%) was observed in the fruit coated with OA (2.0 mM) alone compared to control (3.96%) and all other treatments for 56 days cold stored fruit (Table 8.3). Similarly, Tareen, (2011) has also reported that OA (4.0 mmol) significantly reduced weight loss in 'Flordaking' peach fruit. However, the exact mechanism of reduction of fruit weight loss during cold storage with the application of OA is yet to be investigated. Whilst, when averaged over temperature, the lowest mean weight loss (4.06%) was observed in the fruit coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) compared to control (10.90%) and all other treatments in 84 days cold stored fruit. In the present study, the positive effect of coating such as chitosan, SA and OA alone and combination with chitosan was noted in reducing the loss of weight in cv. Midknight Valencia orange fruit. Ribeiro et al. (2007) reported that edible coatings act as barriers thereby restricting water transfer and protecting fruit skin from mechanical injuries, as well as sealing small wounds and thus delaying dehydration. Possibly, chitosan, SA and OA coating act as a barrier to moisture loss which reduces loss in weight of 'Midknight Valencia' sweet orange during storage at 3°C and 7°C for 56 and 84 days, respectively. Similarly, the reduction of weight loss with coating with chitosan has been reported on litchi (Dong et al., 2004), tomatoes (El Ghaouth et al., 1992b), longan (Jiang and Li, 2001), banana and mango (Kittur et al., 2001), strawberries (Ribeiro et al., 2007), and plum (Bal, 2013).

8.4.4. Firmness

Fruit firmness is one of the important indicators of fruit quality. In the present study, fruit firmness was significantly ($P < 0.05$) higher when fruit were coated with chitosan emulsion (1.5%) alone as compared to control and all other treatments

during storage of fruit for 56 days cold storage, 56 days cold storage followed by 10 days simulated shelf conditions, 84 days cold storage and 84 days cold storage followed by 10 days simulated shelf conditions (Table 8.4). Ethylene also plays an important role in fruit ripening (Bleecker, 2000) and accelerates softening in citrus fruit (Ladaniya, 2007). Softening is coupled with the ripening process and is associated with biochemical changes in cell wall fractions involving hydrolytic processes resulting in breakdown of cell-wall polymers such as cellulose, hemicelluloses and pectins (Payasi et al., 2009). Manganaris et al. (2005a) reported that fruit softening is associated with the increased activities of cell wall-modifying enzymes such as polygalacturonase and pectin esterase. The treatment of chitosan alone and loaded with SA suppressed the ethylene production in 'Midknight Valencia' fruit and thereby retarded the loss of fruit firmness (Gonzalez et al., 2004). Recently, Hussain and Singh (2015) reported that ethylene plays an important role in softening of sweet orange fruit cv. Washington Navel and Lane Late by regulating the activities of softening enzymes (PE, EGase, exo-PG and endo-PG). Similar findings were observed on 'Beijing' peaches with application of SA (Wang et al., 2006), tomato (El Ghaouth et al., 1992b), peach, Japanese pear, kiwifruit (Du et al., 1997), 'Murcott' tangor (Chien et al., 2007), papaya (Ali et al., 2011) and guava (Keqian et al., 2012).

8.4.5. SSC, TA and SSC:TA ratio

The edible coating with chitosan has previously been reported to have a significant effect on the reduction of SSC and TA value in papaya and nectarine by retarding the ripening processes (Asgar et al., 2011; Chiabrando and Giacalone, 2013). Higher SSC:TA ratio in 'Midknight Valencia' orange fruit was recorded in the fruit coated with the chitosan emulsion (1.5%) alone for 56 days cold storage and 56 days cold storage followed by 10 days simulated shelf conditions of storage and may be attributed to reduced level of acidity and higher SSC. The beneficial effects on SSC/TA ratio of edible coatings such as chitosan have been previously reported on different fruit such as peaches (Li and Yu, 2001; Maftoonazad et al., 2008), raspberry and strawberry (Han et al., 2004), nectarine (Chapter, 5), plum (Chapter, 7) and navel oranges (Hu et al., 2013).

8.4.6. Vitamin C

A higher level of vitamin C (35.46 mg 100 mL^{-1} FJ) was noted in the 'Midknight Valencia' sweet orange fruit after 84 days of cold storage, which were coated with 1.5% chitosan emulsion loaded with 2.0 mM SA. Meanwhile, higher level of vitamin C (31.13 mg 100 mL^{-1} FJ) was observed when the fruit were coated with 2.0 mM OA alone during storage for 84 days cold storage followed by 10 days simulated shelf conditions (Table 8.7). It has been reported that edible coatings of chitosan inhibit the activities of vitamin C oxidases (ASA-POD), polyphenol oxidase (PPO), peroxidase (POD) and polygalacturonase (PG) (Ruoyi et al., 2005). Similarly, Srinivasa et al. (2002) and Sritananan et al. (2005) stated that edible coatings reduce the permeability of O_2 in the fruit and that this also delays oxidation of vitamin C. Similarly, edible coatings such as chitosan, SA and OA have reduced vitamin C loss and have been reported previously in nectarine and plum as described in Chapter 4 and 6.

8.4.7. Total antioxidants

Higher levels of total antioxidants were observed in the OA treated 'Midknight Valencia' orange fruit compared to control and all other treatment for storage periods of 56 days cold storage, 56 days cold storage followed by 10 days simulated shelf conditions, 84 days cold storage and 84 days cold storage followed by 10 days simulated shelf conditions (Table 8.8). These changes in the levels of total antioxidants seem to be also influenced by storage period in sweet orange fruit. The beneficial effect of chitosan (0.5%) has been previously reported on apricot fruit during cold storage (Ghasemnezhad et al., 2010). Similarly, higher levels of total antioxidants have been reported in different fruit treated with SA or OA such as peach fruit (Zheng et al., 2007a; Tareen, 2011; Khademi and Ershadi, 2013), papaya (Setha et al., 2000), sugar apple fruit (Mo et al., 2008), grapes (Asghari et al., 2013), peach (Khademi and Ershadi, 2013), mandarin (El-hilali et al., 2003), and oranges (Hu et al., 2013). The exact mechanism of chitosan, SA and OA of influencing levels of total antioxidants in sweet orange fruit is not yet known and warrants investigation.

8.4.8. Chilling injury and disease incidence

Edible coatings have been traditionally used to improve food appearance and maintain quality because they are considered eco-friendly (Khwaldia et al., 2004).

However, the main problems in citrus during postharvest storage are weight loss due to transpiration, chilling injury, ethanol production and diseases (Bruemmer, 1989; Perez-Gago et al., 2002). Coatings can protect citrus from weight loss and chilling injury; however, if used in higher concentrations they can also exacerbate anaerobic conditions during storage. However, the treated fruit of 'Midknight Valencia' sweet orange showed lower chilling injury than control fruit when fruit was stored at 3°C for 56 days cold storage followed by 10 days simulated shelf conditions and 3°C and 7°C for 84 days cold storage followed by 10 days simulated shelf conditions of storage (Table 8.10 and Table 8.11). Similarly, these fruit coated with chitosan (1.5%) showed lower disease incidence than the control and all other treatments at 7°C after 84 days cold storage followed by 10 days simulated shelf conditions of storage (Fig. 8.2). Zhang et al. (2011) reported that chitosan application may possibly have inhibited the germination of fungal spores and mycelium growth on the fruit surface. Similarly, the reduction in chilling injury and incidence of disease with the application of chitosan, SA and OA alone have been reported previously in different fruit such as nectarine fruit (Asghari and Aghdam, 2010), plum (Asghari and Aghdam, 2010; Khademi and Erashadi, 2013), peaches, pears, apples, nectarines and bananas (Mo et al., 2008).

8.5. Conclusion

The treatments of chitosan emulsion SA (2.0 mM) or OA (2.0 mM) alone were more effective than the chitosan loaded with SA or OA and the control by suppressing respiration rate, higher fruit firmness, total antioxidants and reducing disease incidence during cold storage conditions in sweet orange fruit, therefore the proposed hypothesis that chitosan emulsion loaded with SA or OA is more effective than chitosan emulsion, SA and OA individual is refuted. The effect of nanoemulsion of chitosan alone and loaded with SA or OA on extending storage life and maintaining quality of sweet orange fruit may be worth investigating.

CHAPTER 9

General discussion, conclusions and future research

9.1. Introduction

Consumers mostly assess the quality of fresh fruit at the time of purchasing, considering appearance, smoothness, firmness, colour, gloss, aroma and taste (Kader and Siddiq, 2012; Hussain, 2014). However, fruit quality is a major concern to the producers and domestic as well as international consumers. After harvest, fruits and vegetables are prone to physiological and microbiological decay. Several post-harvest treatments have been used to alleviate chilling injury and decay of fruit (Ben-Yehoshua et al., 1987, 1989; Wild, 1990) by different postharvest techniques such as heat shock (Rab and Saltveit, 1996), anaerobic shock treatments (Pesis et al., 1994), chemical treatments, packaging and waxing (Petracek et al., 1999). Fungicides have been used for a long time to control postharvest diseases. However, consumers are worried over the indiscriminate use of fungicides on fruits which is associated with adverse effects on human health and the development of pathogen resistance to fungicides (Stefano et al., 2009; Ren and Shaoying, 2013). Development of alternative methods to conventional usage of fungicides in controlling postharvest diseases in fruit need to be investigated to overcome the concerns of consumers and prevent the development of resistance to fungicides by different pathogens. Traditionally, edible coatings have been tested in the fresh fruits industry as a method to maintain the quality and prolong shelf-life of fresh fruits by minimising microbial spoilage, decreasing moisture loss, respiration and oxidative reaction rates, as well as by reducing physiological disorders (Baldwin et al., 1996; Park, 1999). Similarly, edible coatings have an ability to carry active compounds such as antimicrobial, nutrients, spices flavours, anti-browning agents, and colourants that might help prolong product shelf life and decrease the hazard of microbial growth on food surfaces (Pranoto et al., 2005). Chitosan has an ability to be combined with other compounds such as essential oils or diluted solutions of organic acids such as acetic, propionic, lactic, and glutamic acid in order to enhance its efficacy in extending postharvest life and maintaining quality of horticultural produce (Wilson and El-Ghaouth, 2002; Wilson et al., 2003). In addition, salicylic acid (SA) and OA (OA) have been reported to reduce postharvest losses and maintain quality of

horticultural produce (Asghari and Aghdam, 2010: Cefola and Pace, 2015). Therefore, it was hypothesized that coating application of chitosan loaded with SA or OA will be more effective compared to chitosan, SA or OA alone and in suppressing ethylene production, respiration rate and maintaining postharvest fruit quality of nectarine *(Prunus persica* L. Batch. cv. Honey Fire and Bright Pearl), Japanese plums (*Prunus salicina* Lindl. cv. Angelino and Tegan Blue) and sweet orange (*Citrus sinensis* L. Osbeck cv. Midknight Valencia).

9.2. Effects of chitosan emulsion, salicylic acid or oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid on postharvest quality of nectarine (*Prunus persica* L. Batch. cv nectarine) fruit at ambient temperature.

The edible coatings tested in the experiment showed significant effect on the physico-chemical and physiological properties of the 'Honey Fire' and 'Bright Pearl' nectarine fruit. The effect of coating treatments on nectarine fruit ripening at ambient conditions is genotype dependent. The chitosan coatings are well known to modify gaseous composition around the fresh fruit and vegetables which reduces loss of moisture and reduces decay during storage thus maintaining appearance, shelf life and fruit quality during the postharvest handling stage (Baldwin et al., 1995; Petersen et al., 1999; Romanazzi et al., 2003; Cha and Chinnan, 2004; Valverde et al., 2005). Experimental data presented in this thesis show that postharvest application of chitosan (1.5%) emulsion alone and loaded with SA (2.0 mM) significantly ($P \leq 0.05$) suppressed the mean climacteric ethylene production during ripening of 'Honey Fire' and 'Bright Pearl' nectarine fruit respectively (Fig. 4.1A and B) and lowered fruit decay during ripening of 'Honey Fire' and 'Bright Pearl' nectarine fruit at ambient temperature. Similarly, Noh, (2005) also claimed that chitosan coating acts as an ethylene inhibitor which reduced the activities of key ethylene biosynthesis enzymes such as ACC oxidase and ACO synthase and promotes the storage life of the fresh fruit. Similar effects of chitosan were also previously observed in tomatoes (El Ghaouth et al., 1992b), plum (Wu et al., 2011), jujube (Wang et al., 2009), nectarine (Chapter 4 and 5), plum (Chapter 6 and 7) and sweet oranges (Chapter 8).

Fruit weight loss and fruit firmness is mainly linked with respiration and moisture loss through the fruit skin. Edible coatings may also have a positive effect on fruit weight loss and fruit firmness. Similarly, the positive effect of chitosan

coating in reducing the weight loss of nectarine fruit was recorded in the present study at ambient temperature (Fig. 4.3). Possibly, the chitosan emulsion coating may have acted as a barrier to moisture loss by closing small wounds on the fruit surface and thereby delaying dehydration (Ribeiro et al., 2007). Similar findings have been reported previously in tomato (El Ghaouth et al., 1992b), longan fruit (Jiang and Li, 2001), banana and mango (Kittur et al., 2001), strawberries (Ribeiro et al., 2007) and plum (Bal, 2013). Fruit firmness is also a critical quality characteristic in the consumer acceptability of fresh fruit and vegetables. Nectarine is one of the important soft fruit which suffers a rapid loss of firmness during ripening which contributes greatly to its short postharvest life and susceptibility to fungal contamination. In the present thesis, higher firmness was recorded in the fruit coated with the chitosan emulsion (1.5%) loaded with 2.0 mM SA (2.20-fold) and 2.0 mM chitosan alone (1.88-fold) than the control for 'Honey Fire' nectarine fruit on the third day after treatment at ambient temperature (Fig 4.4). Khan and Singh (2007a) also previously reported that ethylene plays an important role in softening of fruits by regulating the activities of softening enzymes (PE, EGase, exo-PG and endo-PG). In the present thesis, chitosan coating, SA and OA suppressed the endogenous ethylene production in fruit, and possibly the chitosan, SA and OA improved the nectarine fruit firmness via their suppression of the endogenous ethylene (Chapter 4). Similarly, Wang et al. (2006) also reported higher flesh firmness of 'Beijing' peaches treated with SA.

Edible coatings are one of the important methods for improving shelf life and preserving quality of fruit and vegetables because they are considered eco-friendly (Khwaldia et al., 2004). However these coatings act as barriers to moisture and oxygen during processing, handling and storage (Xu et al., 2007). Higher levels of SSC, TA and SSC:TA were noted in the present study after seven days of treatment with chitosan emulsion (1.5%) loaded with 2.0 mM SA and 2.0 mM OA in the 'Honey Fire' nectarine fruit at ambient temperature (Fig. 4.5 and 4.6). Likewise, Li and Yu (2001) and Maftoonazad et al. (2008) claimed that chitosan coated peaches exhibited a decreased loss of acidity.

In the present study different types of organic acids such as citric acid, malic acid, fumaric acid, tartaric acid and succinic acid were determined using a HPLC**.** The results of the present study showed that dominant organic acids in the 'Honey Fire' and 'Bright Pearl' nectarine fruits are citric acid and malic acid followed by

fumaric acid, tartaric acid and succinic acid at ambient temperature (Chapter 4). However, the 'Honey Fire' nectarine fruit treated with chitosan emulsion loaded with OA showed significantly highest mean levels of citric acid (0.35 g $100g^{-1}$ FJ) which suggest an effect of this coating treatment in reducing metabolic activities (Jitareerat et al., 2007). The findings of the present study were also confirmed by the results of (Le Dantec et al., 2010; Wu et al., 2011; Flores et al., 2012) who reported higher level of citric acid, fumaric acid, tartaric acid and succinic acid in different *Prunus* fruits. The dominant sugar in nectarine fruit was sucrose followed by fructose and glucose. Previously published reports highlight that sucrose, fructose and glucose are the major sugar components in stone fruit (Gross and Sams, 1984; Kovács and Németh-Szerdahelyi, 2002; Sozzi, 2004; Ledbetter et al., 2006; Cantín et al., 2009). At the early stage of fruit development the organic acids accumulate in the fruit which is reflected in their acidic taste (Shiratake and Martinoia, 2007). However, at the maturation and ripening stages sugars accumulates in the vacuoles with a simultaneous decrease in organic acids (Yamaki, 1984; Echeverria and Burns, 1989). The results of the present study indicated a higher level of individual sugars when nectarine fruit were stored at ambient temperature (Chapter 4). The findings of the present study were supported by those of Abbasi et al., (2009) who observed that concentration of total sugars in the fruit increases with the advancement of fruit ripening. Similarly, Tareen, (2011) also reported that unripe fruit accumulate starch which converts into sugars during the ripening period.

Highest concentration of vitamin C (14.75 mg 100 mL^{-1} FJ) was noted in the ripe 'Honey Fire' nectarine fruit after seven days of treatment with the chitosan emulsion loaded with SA treatment at ambient temperature (Fig. 4.10). Edible coatings reduce the permeability of O_2 and CO_2 (Srinivasa et al., 2002). Recently, Tareen, (2011) also reported that peach fruit coated with SA or OA alone showed higher level of vitamin C. Similar observations have also been reported on pomegranate fruit (Sayyari et al. 2010), mango (Abbasi et al., 2009), nectarine (Chapter 4) and plum (Chapter 6). All the ripe fruit of nectarine cv. Honey Fire and 'Bright Pearl' coated with chitosan, SA and OA showed significantly higher level of total antioxidants when stored at ambient temperature than control (Fig. 4.11). However, significant effect of SA and OA coatings has also been previously noted in peach fruit (Zheng et al., 2007a; Tareen, 2011; Khademi and Ershadi, 2013), papaya (Setha et al., 2000), mandarin (El-hilali et al., 2003), sugar apple fruit (Mo et al.,

2008) and grapes (Asghari et al., 2013). These observations are in agreement with the findings of the current study where higher levels of total antioxidants have been noted in the SA treated 'Honey Fire' and 'Bright Pearl' nectarine fruit compared to control and other treatments (Fig. 4.11). Whilst, the exact mechanism by which chitosan, SA and OA influence levels of total antioxidants in nectarine fruit is not known and warrants investigation.

Disease incidence in fruit has been reported to be associated with the higher activities of fungal spores and mycelium growth on the fruit surface. Chitosan coating has been shown to inhibit the germination of fungal spores and mycelium growth on the fruit surface by activating pathogen-related (PR) gene function, such as chitinases, chitosanase, β-glucanases, lignin and callose as a defence response in the fruit tissue (Zhang et al., 2011). In the present thesis, the disease incidence percentage was significantly lower, when nectarine fruit 'Bright Pearl' was coated with chitosan emulsion (1.5%), SA (2.0 mM) alone or the chitosan emulsion loaded with the SA (2.0 mM) or OA (2.0 mM) as compared to the control (Fig. 4.12). Similarly, the beneficial effect of SA in reducing percentage disease incidence in different fruit has been previously reported on plum (Khademi and Ershdi, 2013; Asghari and Aghdam, 2010) and in peaches, pears, apples, nectarines and bananas (Mo et al., 2008). The present study was supported by the findings of Romanazzi et al. (2003), Bal, (2013) and Bautista-Banos et al. (2006) who observed that chitosan could effectively inhibit postharvest diseases on various horticultural commodities.

In conclusion, the postharvest application of chitosan (1.5%) emulsion loaded with SA (2.0 mM) or OA (2.0 mM) were effective in maintaining most of the quality parameters as compared to other treatments and control in 'Honey Fire' nectarine. However, in cv. Bright Pearl, a coating of chitosan alone was more effective in maintaining various fruit quality parameters compared to all other treatments and control at ambient temperature. The proposed hypothesis that chitosan loaded with SA or OA is more effective compared to chitosan, SA and OA alone was supported by the findings of the present study only in 'Honey Fire' nectarine, whilst in cv. Bright Pearl, the proposed hypothesis was rejected.

9.3. Influence of chitosan emulsion, salicylic acid or oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid on cold storage life and fruit quality of nectarine (*Prunus persica* L. Batsch. cv nectarine)

In this experiment, the effects of different postharvest coatings such as chitosan emulsion, SA or OA alone and chitosan emulsion loaded with SA or OA on cold storage life and quality of nectarine fruit cv 'Bright Pearl' were investigated. The experimental data for this thesis showed that postharvest coatings of SA alone and SA loaded into chitosan suppressed the climacteric ethylene production in four week cold stored 'Bright Pearl' nectarine fruit as compared to control and all other treatments (Chapter 5). The findings of the present study was supported by earlier findings of jujube fruit (Wang et al., 2009), peach, pear, apple (Mo et al., 2008), strawberry (Shafiee et al., 2010; Vu et al., 2011), peach (Li and Yu, 2001), and papaya (Asgar et al., 2011). Recently, Huang et al. (2013) also reported that OA suppressed the ethylene production and delayed climacteric ethylene peak in banana fruit during cold storage. Similarly, edible coatings of chitosan, SA, OA alone and chitosan loaded with SA and OA significantly reduced fruit weight loss and maintained fruit firmness by suppressing the endogenous ethylene production in the cultivar of nectarine at cold storage in the present study (Chapter 5). The reduction in the fruit weight loss with different coating treatments reported here is possibly due to reduced transpiration from the fruit during cold storage as also reported previously in peach (Tareen, 2011), tomato (El Ghaouth et al., 1992b), longan fruit (Jiang and Li, 2001), banana and mango (Kittur et al., 2001) and strawberries (Ribeiro et al., 2007). In this thesis, higher firmness was recorded in the fruit coated with chitosan emulsion alone (46.38 N), SA alone (45.56 N) and the chitosan emulsion loaded with SA (42.60 N) compared to the control 'Bright Pearl' nectarine fruit (Table 5.1A). Possibly, the reduction in nectarine fruit firmness due to these coating treatments may be ascribed to the reduced activities of various fruit softening enzymes. Similarly, Manganaris et al. (2005a) previously reported fruit softening in nectarine fruit is related to the higher activities of cell wall-modifying enzymes such as polygalacturonase and pectin esterase.

All the edible coating treatments of chitosan, SA, OA alone and chitosan loaded with SA and OA increased firmness, levels of fructose, fumaric acid, succinic acid, total organic acids and total antioxidants in the fruit juice in 'Bright Pearl' nectarine as compared to the control fruit during cold storage in this thesis (Chapter

5). The increase in these parameters may possibly be ascribed to the treatments acting as barriers to moisture and oxygen during processing, handling and storage of the fresh fruit (Xu et al., 2007). The beneficial effects of edible coatings with chitosan and SA alone on maintaining fruit quality have been reported on various different fruits such as peach (Li and Yu, 2001), strawberry (Vu et al., 2011) and papaya (Asgar et al., 2011). The exact mechanism by which chitosan, SA and OA influence levels of total antioxidants and regulate metabolism of sugars and organic acids in nectarine fruit during cold storage is not known and warrant investigation. In contrast, in this thesis, nectarine fruit which were coated with chitosan emulsion (1.5%), the chitosan emulsion loaded with SA and SA 2.0 mM alone or the chitosan emulsion loaded with OA 2.0 mM exhibited significantly lower percentage disease incidence compared to the control and the treatment of OA alone (Fig. 5.3) when all fruit were stored for four weeks at temperature 0-1°C. Previous studies have also reported that chitosan alone could effectively inhibit postharvest diseases in various horticultural crops during storage (Romanazzi et al., 2003; Bautista-Banos et al., 2006; Zhang et al., 2011; Bal, 2013). Recently, Khademi and Ershdi, (2013) also reported that SA treatment reduced fruit decay in plum fruit. In conclusion, the 'Bright Pearl' nectarine fruit coated with chitosan emulsion, SA or OA alone was more effective in maintaining quality in four weeks cold stored fruit compared to chitosan emulsion loaded with SA or OA. The proposed hypothesis that chitosan loaded with SA or OA is more effective than chitosan, SA and OA alone in maintaining quality of cold stored 'Bright Pearl' nectarine fruit is rejected.

9.4. Postharvest quality of Japanese plums (*Prunus salicina* Lindl. cv Angeleno and Tegan Blue) fruit at ambient temperature influenced by coating of chitosan, salicylic acid or oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid

In the present study plum fruit cv. Angelino and 'Tegan Blue' coated with chitosan emulsion (1.5%) and OA (2.0 mM) alone exhibited significantly ($P \leq 0.05$) suppressed and delayed climacteric ethylene production compared to the control and other treatments during ripening period (Fig. 6.1A and 6.2 A and Fig. 6.1B and 6.2 B). The suppressed endogenous ethylene production in chitosan coated plum fruits may be ascribed to the hindrance of the entry of oxygen into the plum (Noh, 2005) because ethylene biosynthesis is dependent on the presence of O_2 (Abeles et al.,

1992). It may also be possible that chitosan coating suppressed endogenous ethylene production by retarding the activities of key ethylene biosynthesis enzymes such as ACC oxidase and ACO synthase (Noh, 2005). The findings of the current study are also supported by studies on different fruits such as tomatoes, cucumbers and bell peppers (El Ghaouth et al., 1992b) and plum (Abdi et al., 1998; Khan and Singh, 2007b; Wu et al., 2011).

In the present study, the beneficial effect of chitosan coating was observed in reducing the loss of weight in cv. Angelino and 'Tegan Blue' of plums (Fig. 6.3A and B). The results were supported by the findings of Ribeiro et al. (2007) who claimed that possibly, edible coatings act as barriers, thereby restricting water transfer and protecting fruit skin from mechanical injuries, as well as sealing small wounds and thus delaying dehydration. Similar results of chitosan coatings have been observed on litchi (Donglin et al., 1997; Dong et al., 2004), tomatoes (El Ghaouth et al., 1992b), longan fruit (Jiang and Li, 2001), banana and mango (Kittur et al., 2001), strawberries (Ribeiro et al., 2007), and plum (Bal, 2013).

The fruit firmness was significantly ($P \leq 0.05$) higher in both 'Angelino' and 'Tegan Blue' cultivars of plum when fruit coated with chitosan emulsion alone and loaded with SA as compared to control and all other treatments and may be ascribed to the reduced ethylene production (Fig. 6.4A and B). Earlier, Khan and Singh (2007a) reported a substantial reduction of plum fruit softening with the exogenous application of 1-methylcylclopropene, an ethylene antagonist. Similarly, beneficial effects of chitosan on reduction of loss of fruit firmness in different fruits have been reported such as in peach, Japanese pear, kiwifruit (Du et al., 1997) and citrus 'Murcott' tangor (Chien et al., 2007) and plum (Chapter 6).

'Angelino' plum fruit when coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) showed higher SSC and TA compared to the control and all other treatments (Fig. 6.5A and Fig 6.6A). Higher SCC in coated fruit may be ascribed to reduced metabolic rate compared to the control fruit. Similar effects of chitosan on peaches (Li and Yu, 2001; Maftoonazad et al., 2008), litchi (Dong et al., 2004) and nectarine fruit (Chapter, 4) have also been reported.

Organic acid and sugars are major components of fruit quality. Malic acid is the main organic acid present in plum fruit (Le Dantec et al., 2010; Wu et al., 2011). In the

present study citric acid, tartaric acid and succinic acid have also been estimated in both cultivars of plum (Chapter 6). Malic acid was predominant followed by succinic acid, tartaric acid, fumaric acid and citric acid among different organic acids in the 'Angelino' and 'Tegan Blue' plum fruit. Similarly, fructose was a dominant sugar in plum fruit followed by glucose and sucrose (Chapter 6). Similarly, sucrose, fructose and glucose have been reported as major sugar components in various stone fruits (Gross and Sams, 1984; Németh-Szerdahelyi, 2002; Sozzi, 2004; Kovács and Ledbetter et al., 2006; Cantín et al., 2009).

Higher concentration of vitamin C was noted in cv. Tegan Blue when fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) and SA (2.0 mM) alone respectively compared to control and all other treatments (Fig. 6.12B). It has been previously reported that edible coatings reduce the permeability of O_2 and CO_2 in the fruit (Srinivasa et al., 2002) and thus delay the oxidation of vitamin C (Sritananan et al., 2005). Similarly, the effect of chitosan of higher levels of vitamin C has been observed in peach (Ruoyi et al., 2005; Tareen, 2011) and mango (Abbasi et al., 2009). Higher level of total antioxidants (46.26 µM Trolox 100 ml-1 FJ) was observed in cv. Tegan Blue when coated with OA (2.0 mM) as compared to control and all other treatments (Fig. 6.13B). However, lowest level of total antioxidants (41.96 µM Trolox 100 ml-1 FJ) was observed in cv. Tegan Blue when fruit were coated with SA (2.0 mM) compared to control and all other treatments. Increased levels of antioxidants have also been reported in different fruit coated with chitosan emulsion, such as apricot (Ghasemnezhad et al., 2010), sugar apple fruit (Mo et al., 2008) and grapes (Asghari et al., 2013), and recently in our study on nectarine (Chapter 4). However the exact mechanism of chitosan, SA and OA of influencing levels of total antioxidants in plum fruit is not known and warrants investigation. In the present study, the lowest percentage of disease incidence (7.5%) was recorded when fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) as compared to control and all other treatments in 'Tegan Blue' plum fruit (Fig 6.14B). The current research findings were supported by the findings of Asghari and Aghdam, (2010) on nectarine fruit and Mo et al. (2008) on peaches, pears, apples, and bananas. Within this thesis, the work of this research on nectarine (Chapter 4 and 5) also support the current findings on plum (Chapter 6). Coatings can retard food deterioration by inhibiting the growth of microorganisms, due to their natural

intrinsic activity or to the incorporation of antimicrobial compounds (Cha and Chinnan, 2004).

In conclusion, postharvest application of chitosan emulsion alone was more effective in down regulating the ethylene production in both 'Tegan Blue' and Angelino' plum, whilst, chitosan emulsion loaded with SA was more effective in maintaining fruit quality of plum cultivar 'Tegan Blue' at ambient temperature compared to uncoated fruit and other treatments. The proposed hypothesis that chitosan loaded with SA or OA is more effective than chitosan, SA and OA alone in maintaining fruit quality of 'Tegan Blue' plum was supported. In cultivar 'Angelino', chitosan emulsion and OA alone treatments were more effective in suppressing ethylene production and chitosan emulsion alone coating was more effective in maintaining fruit quality at ambient temperature. The proposed hypothesis that chitosan loaded with SA or OA is more effective than chitosan, SA and OA alone in supressing ethylene production and maintaining fruit quality of 'Angelino' plum was rejected.

9.5. Impact of chitosan emulsion, salicylic acid or oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid on postharvest quality of cold stored Japanese Plum (*Prunus salicina* Lindl. cv Angelino and Tegan Blue) fruit

Most plum cultivars are climacteric fruit which are highly perishable and cold storage is recommended to extend fruit shelf-life as well as maintain the fruit quality (Crisosto et al., 2004). However, though commercial storage conditions and transportation facilities delay fruit softening and reduce weight loss and disease incidence, they may also lead to development of cold storage disorders such as chilling injury (CI) (Crisosto et al., 2008; Singh and Singh, 2008). Therefore, appropriate postharvest techniques combined with cold storage are necessary to maintain the quality of fresh fruit of plum. The current experiment was designed to evaluate the combined effect of cold storage and edible coating on ethylene production, disease incidence and fruit quality of plum. In the current study it was observed that chitosan emulsion (1.5%) coating, SA, OA alone and chitosan emulsion (1.5%) loaded with SA or OA significantly ($P \leq 0.05$) suppressed climacteric ethylene production compared to the control in 'Angelino' and 'Tegan Blue' plum fruit during cold storage period (Table 7.1). Similarly, chitosan coating

has previously been reported to suppress ethylene production in different fruits such as tomatoes, cucumbers and bell peppers (El Ghaouth et al., 1992b). Wu et al. (2011) also observed the reduction in ethylene production in 'Damili' plum fruit treated with 5.0 mM OA.

Edible coatings have also been studied in relation to spoilage, especially chilling injury and browning in different fresh fruit and vegetables. Prevention of spoilage has sometimes been attributed to the physical barrier of coatings hindering O_2 and CO_2 diffusion which decreases respiration rate (Erbil and Muftugil, 1986). In the present study, coating of chitosan emulsion loaded with SA and chitosan emulsion alone reduced the loss of weight in both 'Angelino' and 'Tegan Blue' cultivars of plums respectively (Table 7.3 and Table 7.4) possibly reducing the moisture loss from the fruit surface. Edible coatings such as chitosan, SA and OA act as barriers, thus restricting water transfer and protecting plum fruit skin from mechanical injuries, as well as sealing small wounds and thus delaying dehydration. In this thesis, the experimental findings were also supported by previous findings that edible coating reduced weight loss of different fruits such as litchi (Donglin et al., 1997; Dong et al., 2004), tomato (El Ghaouth et al., 1992b), longan fruit (Jiang and Li, 2001), banana and mango (Kittur et al., 2001), strawberries (Ribeiro et al., 2007), and plum (Bal, 2013). Firmness is one of the important fruit quality parameters. In the current study, the fruit firmness was found to be higher in both 'Angelino' and 'Tegan Blue' cultivars of plum with fruit coated with chitosan emulsion loaded with SA as compared to control and all other treatments which may be ascribed to the reduced ethylene production (Table. 7.5 and Table 7.6). Ethylene is known to promote the activity of various fruit softening enzymes such as PE, EGase, exo-PG and endo-PG in plum cv. Tegan Blue (Khan and Singh, 2007a). However, the reduction of loss of fruit firmness with the application of chitosan has also been previously reported in different fruits such as peach, Japanese pear, kiwifruit (Du et al., 1997) and citrus 'Murcott' tangor (Chien et al., 2007) and mango and pears (Zhu et al., 2008).

All of the treatments of edible coatings with chitosan, SA, OA and chitosan loaded with SA and OA improved SSC, vitamin C, total antioxidants, the individual sugars and the total sugars as well as individual organic acid in the fruit juice in both cultivars of plum during cold storage (Chapter 7). However, in the present study

'Angelino' and 'Tegan Blue' plum fruits when coated with OA (2.0 mM) showed higher SSC and SSC: TA ratio respectively compared to the control and all other treatments (Table. 7.5 and Table 7.6). Similar effects of chitosan have also been reported previously on peaches (Li and Yu, 2001; Maftoonazad et al., 2008), litchi (Dong et al., 2004), nectarine fruit (Chapter 4 and 5) and plum (Chapter 6 and 7). Chitosan application also improved the individual and total sugars as well as organic acid in the plum during cold storage in the present study (Chapter 7). These findings are also in accordance with the findings of Shiratake and Martinoia, (2007), Yamaki, (1984) and Echeverria and Burns, (1989) and Abbasi et al. (2009). However, in the present study, significantly higher concentration of vitamin C (8.35 mg 100 ml^{-1} FW) was noted in cv. Tegan Blue when fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) compared to control and all other treatments (Table 7.12). It has been previously reported that edible coatings reduce the permeability of O_2 and CO_2 in the fruit (Srinivasa et al., 2002) and thus can delay the oxidation of vitamin C (Sritananan et al., 2005). The current study was also supported by the findings of Abbasi et al. (2009) who observed higher levels of vitamin C in mango fruit coated with chitosan. Likewise, in the present study a higher level of total antioxidants was observed in both 'Angelino' and 'Tegan Blue' cultivars respectively when coated with SA (2.0 mM) as compared to control and all other treatments (Table 7.13 and Table 7.14). Previously, increases in antioxidants have been reported in plum cv. 'Santa Rosa' fruit after postharvest coatings of SA (Davarynejad et al., 2013), peach (Khademi and Ershadi, 2013), sugar apple fruit (Mo et al., 2008), grapes (Asghari et al., 2013), and as described in this thesis in nectarine (Chapter 4 and 5). The mechanism by which chitosan, SA and OA influences levels of total antioxidants in cold stored plum fruit is not known and warrants investigation. Chitosan coating has previously been reported to reduce weight loss and sensory quality, with higher soluble solids concentration, titratable acid, and vitamin C by suppressing the activities of polyphenol oxidase (PPO) and peroxidase (POD) in litchi fruit (Dong et al., 2004). The results of the present study showed lowest disease incidence when plum fruit were coated with chitosan emulsion (1.5%) alone as compared to control and all other treatments in 'Angelino' fruit during cold storage for eight weeks (Fig. 7.1A). Meanwhile, lowest disease incidence was recorded when fruit were coated with chitosan emulsion (1.5%) loaded with SA (2.0 mM) as compared to control and all other treatments in 'Tegan Blue' plum fruit during six weeks of cold storage (Fig.

7.1B). The findings of the current study were supported by previous reports that SA reduced disease incidence on strawberry fruit (Asghari and Aghdam, 2010), peaches, pears, apples, peach (Khademi and Ershadi, 2013), plum (Davarynjad et al., 2013) and bananas (Mo et al., 2008).

In conclusion, the chitosan emulsion loaded with SA was more effective compared to other treatments in suppressing ethylene production, reducing weight loss and disease incidence, and increasing firmness, TA and vitamin C in 'Tegan Blue' cultivar. Whilst, the chitosan emulsion alone was more effective in suppressing ethylene production, reducing disease incidence, higher TA, total organic acids and sugars and vitamin C in cv. Angelino plum fruit as compared to all other treatments. The hypothesis that chitosan emulsion loaded with SA or OA is more effective than chitosan, SA or OA alone in suppressing ethylene production and maintaining fruit quality of 'Tegan Blue' was supported but it was refuted in 'Angelino' plum fruit following cold storage.

9.6. Effects of chitosan emulsion, salicylic acid or oxalic acid alone and chitosan emulsion loaded with salicylic acid or oxalic acid on postharvest quality of sweet orange (cv. Midknight Valencia) fruit at different temperature

Edible coatings improved the appearance of fruit making the produce more acceptable to the consumers. Keeping in view the importance to increase the post-harvest life of sweet oranges the present studies were carried out to evaluate the effect of edible coatings on physiological characteristics of sweet oranges cv. Midknight Valencia. The fruit of 'Midknight Valencia' coated with chitosan emulsion (1.5%) loaded with OA (2.0 mM) and chitosan loaded with SA (2.0 mM) suppressed ethylene production more than the control and all other treatments at all storage periods except at 56 days cold storage (Fig. 8.1). It has been previously reported that chitosan coatings can delay the ripening of tomatoes (El Ghaouth et al., 1992b). The edible coating can act as a protective barrier on the fruit surface which reduces availability of oxygen and ultimately reduces the fruit respiration rate and extend storage life (Du et al., 1997; El Ghaouth et al., 1991; Jiang and Li, 2001). OA applications have also been reported to reduce ethylene production, respiration rate and maintain fruit firmness in plum (Wu et al., 2011), mango (Zheng et al., 2007) and jujube (Wang et al., 2009).

In the present study, the beneficial effect of coating such as chitosan, SA and OA alone and in combination with chitosan was noted through reduction in the loss of weight in cv. Midknight Valencia orange fruit. Likewise, Ribeiro et al. (2007) reported that edible coatings act as barriers thereby restricting water transfer and protecting fruit skin from mechanical injuries, as well as sealing small wounds and thus delaying dehydration. Similarly, different coating materials have been reported to reduce weight loss in various fruits such as litchi (Dong et al., 2004), longan fruit (Jiang and Li, 2001), banana and mango (Kittur et al., 2001), strawberries (Ribeiro et al., 2007), and plum (Bal, 2013).

In the present study, fruit firmness was significantly higher when the fruit were coated with chitosan emulsion alone as compared to control and all other treatments. Ethylene plays an important role in fruit ripening (Bleecker, 2000) and accelerates softening in citrus fruit (Ladaniya, 2007). Softening is known as a ripening process and associated with biochemical changes in cell wall functions involving hydrolytic processes resulting in breakdown of cell-wall polymers such as cellulose, hemicelluloses and pectins (Payasi et al., 2009). Similar findings have been previously observed on 'Beijing' peaches with application of SA (Wang et al., 2006), tomato (El Ghaouth et al., 1992b), peach, Japanese pear, kiwifruit (Du et al., 1997), 'Murcott' tangor (Chien et al., 2007), papaya (Ali et al., 2011) and guava (Keqian et al., 2012).

Chitosan coating has also been reported to significantly reduce levels of SSC and TA value in nectarine by slowing down the senescence process (Asgar et al., 2011; Chiabrando and Giacalone, 2013). The beneficial effect of edible coatings such as chitosan have been previously reported since SSC and TA showed higher value in different fruit such as peaches (Li and Yu, 2001; Maftoonazad et al., 2008), raspberry and strawberry (Han et al., 2004), nectarine (Chapter 5 of this thesis), plum (Chapter 7 of this thesis) and navel oranges (Hu et al., 2013). Likewise, the present study found higher level SSC in oranges treated with chitosan emulsion, SA and OA alone and chitosan loaded with SA or OA as compared to the control. The fruit coated with chitosan emulsion loaded with SA exhibited higher TA as compared to control and all other treatments for all storage periods except at 56 days cold storage.

In the current study, higher levels of total antioxidants have been noted in the OA treated 'Midknight Valencia' orange fruit compared to control and all other treatments in 56 days cold storage, 56 days cold storage followed by 10 days

simulated shelf conditions, 84 days cold storage and 84 days cold storage followed by 10 days simulated shelf conditions (Table 8.8). Srinivasa et al. (2002) and Sritananan et al. (2005) claimed that edible coatings reduce the permeability of O_2 and CO_2 in the fruit which can delay the oxidation of vitamin C. The effect of edible coatings such as chitosan, SA and OA on suppressing ethylene production, reducing weight loss and disease incidence and maintaining fruit quality has been reported previously on different fruit, for example peach (Ruoyi et al., 2005; Tareen, 2011), mango (Abbasi et al., 2009), pomegranate (Sayyari et al., 2010), litchi (Dong et al., 2004), oranges (Hu et al., 2013), nectarine (Chapter 4 of this thesis) and plum (Chapter 6 of this thesis). It is probable that edible coating inhibited the activities of vitamin C oxidases (ASA-POD), peroxidase (POD), polygalacturonase (PG) and polyphenol oxidase (PPO).

'Midknight Valencia' sweet orange fruit treated with 2.0 mM OA alone showed lower chilling injury than control fruit followed by 2.0 mM SA when fruit was stored at 3°C for 56 days cold storage followed by 10 days simulated shelf conditions and 3°C and 7°C for 84 days cold storage followed by 10 days simulated shelf conditions of storage (Table 8.10 and 8.11). Asghari and Aghdam, (2010) reported that SA application decreased chilling injury in horticultural crops. In the current study, all of the treatments exhibited lower disease incidence as compared to the control. The beneficial effects of chitosan, SA and OA alone in disease incidence have been reported previously on different fruit such as nectarine fruit (Asghari and Aghdam, 2010), plum (Khademi and Erashadi, 2013; Asghari and Aghdam, 2010), peaches, pears, apples, nectarines and bananas (Mo et al., 2008). Zhang et al. (2011) reported that chitosan application possibly may have inhibited the germination of fungal spores and mycelium growth on the fruit surface.

The changes in the levels of total antioxidants in 'Midknight Valencia' orange fruit during storage were found to be significant which suggests that the storage period affects the levels of antioxidants in sweet orange fruit. SA and OA application have been reported to increase activities of antioxidant enzymes on different fruit such as peach fruit (Zheng et al., 2007; Tareen, 2011; Khademi and Ershadi, 2013), papaya (Setha et al., 2000), sugar apple fruit (Mo et al., 2008), grapes (Asghari et al., 2013), peach (Khademi and Ershadi, 2013), mandarin (El-hilali et al., 2003), and oranges (Hu et al., 2013). The exact mechanism by which chitosan, OA and SA influence levels of total antioxidants in orange fruit is not yet known and warrants

investigation. In general, the treatments of chitosan emulsion, SA (2.0 mM) and OA (2.0 mM) alone were more effective than the chitosan loaded with SA or OA in suppressing respiration rate and reducing disease incidence, higher fruit firmness, SSC:TA ratio, vitamin C and total antioxidants during cold storage conditions in sweet orange fruit. Therefore the proposed hypothesis is refuted.

9.7. Conclusions

- Coating of chitosan emulsion loaded with SA or OA were more effective as compared to treatments of chitosan, SA or OA alone and the control in maintaining most of the quality parameters in 'Honey Fire' nectarine fruit kept at room temperature. Meanwhile, chitosan emulsion, SA or OA alone proved better as compared to the chitosan emulsion loaded with SA or OA in maintaining most of the quality parameters in 'Bright Pearl' nectarine fruit kept at ambient temperature. These treatments were tested only on two cultivars of nectarines due to limitation of time; in future more cultivars should be tested using these treatments.
- Edible coatings of chitosan emulsion, SA or OA alone were more efficient as compared to the coating of chitosan emulsion loaded with SA or OA in maintaining quality of four-week cold stored fruit of 'Bright Pearl' nectarine fruit. This experiment was limited to testing one cultivar only.
- 'Angelino' plum fruit coated with chitosan emulsion alone and kept at ambient temperature for two weeks exhibited supressed ethylene production, higher firmness, SCC:TA ratio, total organic acids and sugars, and total antioxidants and lower disease incidence as compared to those coated with chitosan emulsion loaded with SA or OA and all other treatments. Meanwhile, 'Tegan Blue' plum fruit coated with chitosan emulsion loaded with SA and kept at room temperature for two weeks showed reduced weight loss, reduced incidence of disease, and higher total organic acids, sugars and vitamin C compared to the fruit treated with different coatings and control.

- 'Angelino' plum fruit coated with chitosan emulsion alone following an eight week cold storage period showed supressed ethylene production: and higher TA, total organic acids and sugars, and lower disease incidence as compared

to those coated with chitosan emulsion loaded with SA or OA and all other treatments. Meanwhile, 'Tegan Blue' plum fruit coated with chitosan emulsion loaded with SA after six weeks cold storage exhibited supressed ethylene production, reduced weight loss, reduced incidence of disease and higher firmness, TA and vitamin C compared to the fruit treated with different coatings and control.

- 'Midknight Valencia' sweet orange fruit coated with chitosan emulsion loaded with OA showed supressed ethylene production in 56 days cold storage followed by 10 days simulated shelf conditions, 84 days cold storage only and 84 days cold stored fruit followed by 10 days simulated shelf conditions as compared to all other treatments. The fruit coated with chitosan emulsion alone showed higher firmness in the fruit stored for 56, 86 days cold storage and followed by 10 days simulated shelf conditions as compared to all other treatments. The fruit coated with OA alone showed higher levels of total antioxidants and lower chilling injury irrespective of cold storage period followed by 10 days simulated shelf conditions. The disease incidence was lowest in the fruit coated with chitosan emulsion alone and kept in cold storage for 84 days followed by 10 days simulated shelf conditions as compared to all other treatments and control.

9.8. Future research

This research work focused on the role of chitosan emulsion, SA, OA alone and chitosan emulsion loaded with SA or OA on fruit ripening, ethylene biosynthesis, respiration, weight loss, firmness, fruit quality including titratable acidity (TA), soluble solids concentration (SSC), SSC:TA ratio, changes in sugars and organic acids, vitamin C, total antioxidants and disease incidence in the climacteric fruits Japanese plum and nectarine and the non-climacteric fruit sweet orange. However, future research work may be required in the following areas:

- The edible coating application of chitosan emulsion, SA, OA and chitosan emulsion loaded with SA and OA suppressed ethylene production in both non-climacteric fruit (sweet orange) and climacteric fruits (nectarine and plum). The mechanism of how these coatings down regulate ethylene

biosynthesis in climacteric and non-climacteric fruits now warrants investigation.

- Application of chitosan emulsion, SA, OA and chitosan emulsion loaded with SA or OA reduced and delayed the loss of fruit firmness during cold storage period in non-climacteric fruit (sweet orange) and climacteric fruits (nectarine and plum). The mode of action of these coatings in regulating fruit softening process has yet to be investigated.
- Coatings of chitosan emulsion, SA, OA and chitosan emulsion loaded with SA or OA increased levels of vitamin C and total antioxidants during cold storage period in non-climacteric fruit (sweet orange) and climacteric fruits (nectarine and plum). How these coatings modulate the levels of vitamin C and total antioxidants during cold storage period in non-climacteric and climacteric fruit is yet to be elucidated.
- Whether the coatings of chitosan emulsion, SA, OA and chitosan emulsion loaded with SA or OA regulate expression of genes involved in ethylene biosynthesis and fruit softening processes is worthy of investigation in the future.

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