Phospholipase D Inhibition Technology for Enhancing the Shelf Life and Quality of Greenhouse Tomato and Sweet Bell Pepper

By

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ABSTRACT

PHOSPHOLIPASE D INHIBITION TECHNOLOGY FOR ENHANCING THE SHELF LIFE AND QUALITY OF GREENHOUSE TOMATO AND SWEET BELL PEPPER

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From harvest to consumption, fresh produce is exposed to different exogenous factors contributing to product deterioration. Phospholipase D is a key enzyme involved in membrane deterioration that occurs during fruit ripening and senescence. Hexanal, an inhibitor of phospholipase D is known to enhance membrane preservation, and has been successfully applied for pre- and post-harvest treatment of fruits, vegetables and flowers. The current work aims to demonstrate the effectiveness of pre- and post-harvest application of hexanal formulations on quality parameters and shelf life of greenhouse tomatoes and bell peppers. Preharvest spray application of aqueous formulations containing hexanal alone, or in combination with other ingredients such as antioxidants, enhanced firmness, ascorbic acid and soluble solids in the treated fruits. The average fruit weight and yield remained the same in treated and untreated plots. Post-harvest dip application of hexanal formulations also enhanced quality parameters and shelf life of tomato even after 4 weeks of storage at 12°C, followed by one week at room temperature. Our results suggest that preharvest or postharvest application of hexanal formulations can result in enhanced membrane preservation in tomatoes and bell peppers. This observation was further supported by the effect of hexanal vapour treatments on quality parameters, shelf-life and antioxidative enzyme activities of bell pepper fruits. Treated fruits showed an increase in firmness, a reduction in physiological water loss and lowered electrical conductivity; which
indicated better membrane preservation during storage. These treatments also resulted in an increase in the levels of protein concentration and antioxidant enzyme activities, specifically that of superoxide dismutase, catalase, glutathione reductase, guaiacol peroxidase and ascorbate peroxidase. Analysis of the release kinetics of hexanal vapour in a contained system suggested that most of the hexanal applied as vapour was absorbed and metabolized within the tissue of pepper fruits. These studies also revealed that 6 h hexanal vapour exposure is the minimum effective treatment time required for obtaining enhanced preservation of pepper fruits. Using a headspace solid phase microextraction (SPME) method for volatile extraction in peppers, 11 additional compounds were identified in hexanal-treated fruit. Overall, the present studies provide a strong rationale for adopting the hexanal-based technologies to enhance the quality and shelf life of greenhouse produce.
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# TABLE OF CONTENTS

Abstract ............................................................................................................................... ii
Acknowledgements ........................................................................................................ iv
Table of Contents ........................................................................................................... v
List of Tables .................................................................................................................... x
List of Figures ................................................................................................................ xi
List of Abbreviations ..................................................................................................... xiii
Chapter 1  Introduction ................................................................................................. 1
Chapter 2  Literature Review ...................................................................................... 12
  2.1 Fruits and vegetables in human health ............................................................... 12
  2.2 Greenhouse industry in Canada ......................................................................... 14
  2.3 Fruit quality attributes ....................................................................................... 15
     2.3.1 Appearance and colour ............................................................................. 16
     2.3.2 Texture ..................................................................................................... 19
     2.3.3 Flavour .................................................................................................... 23
     2.3.4 Nutritional quality and antioxidant ......................................................... 27
  2.4 Fruit quality deterioration .................................................................................... 32
     2.4.1 Physiological ............................................................................................ 32
        2.4.1.1 Respiration ....................................................................................... 32
           2.4.1.1.1 Temperature .............................................................................. 36
        2.4.1.2 Atmospheric composition ................................................................ 40
        2.4.1.3 Physical stress .................................................................................. 42
     2.4.2 Mechanical ............................................................................................... 50
  2.5 Changes in fruits during ripening and senescence ............................................ 53
  2.6 Changes in membranes of the ripening fruits ................................................... 55
  2.7 Phospholipase D ............................................................................................... 61
  2.8 Phospholipid catabolism ................................................................................... 63
  2.9 Phospholipase D inhibition by hexanal and enhancing shelf life .................... 66
  2.10 Fruit volatiles ................................................................................................. 69
     2.10.1 Volatile compounds of bell peppers ....................................................... 69
     2.10.2 Volatile compounds of tomatoes ............................................................. 72
Chapter 3

Improving quality of greenhouse tomato (*Solanum lycopersicum* L.) by Pre- and postharvest application of hexanal-containing formulations   ........................................................................................................... 77

3.1 Abstract ...................................................................................................................... 77

3.2 Introduction ............................................................................................................... 78

3.3 Materials and methods ............................................................................................ 82

3.3.1 Plant materials ...................................................................................................... 82

3.3.2 Hexanal treatment .................................................................................................. 83

3.3.2.1 Preharvest spray application of hexanal and EFF .............................................. 83

3.3.2.2 Postharvest dip application of hexanal and EFF ............................................. 84

3.3.3 Analysis of quality parameters ............................................................................. 86

3.3.3.1 Colour measurements .......................................................................................... 86

3.3.3.2 Firmness measurements ....................................................................................... 86

3.3.3.3 Ascorbic acid ....................................................................................................... 86

3.3.3.4 Soluble solids (°Brix) .......................................................................................... 87

3.3.3.5 pH measurements ............................................................................................... 88

3.3.3.6 Organic acid ........................................................................................................ 88

3.3.3.7 Effect of hexanal formulations on tomato yield .................................................. 88

3.3.3.8 Effect of hexanal formulations on fruit shelf life ............................................... 88

3.3.4 Statistical analysis ................................................................................................ 89

3.4 Results ....................................................................................................................... 89

3.4.1 Effect of preharvest treatments on quality parameters of tomatoes ..................... 89

3.4.1.1 Colour parameters of fruit .................................................................................... 89

3.4.1.2 Effect of treatments on fruit firmness .................................................................... 91

3.4.1.3 Effect of preharvest treatments on soluble solids (°Brix) ................................. 92

3.4.1.4 Effect of preharvest treatments on ascorbic acid levels ................................... 93

3.4.1.5 Effect of preharvest treatments on organic acids and pH ................................. 94

3.4.1.6 Effect of preharvest treatments on tomato yield .............................................. 95

3.4.2 Effect of postharvest treatments on quality attributes of tomatoes ....................... 96

3.4.2.1 Effect of postharvest treatments on fruit colour ................................................. 96

3.4.2.2 Effect of postharvest treatments on fruit firmness ............................................ 97
3.4.2.3 Effect of postharvest treatments on ascorbic acid levels .......... 98
3.4.2.4 Effect of postharvest treatments on soluble solids and organic acid ................................................................. 98
3.4.2.5 Effect of postharvest treatments on shelf life of tomatoes .......... 99
3.5 Discussion .................................................................................................................................................. 100
3.6 Conclusion ............................................................................................................................................. 105

Chapter 4 Volatile generation and release kinetics of hexanal during postharvest vapour application for enhancing shelflife and quality of bell peppers ...... 106
4.1 Abstract .................................................................................................................................................. 106
4.2 Introduction ........................................................................................................................................... 106
4.3 Materials and methods .................................................................................................................. 110
  4.3.1 Plant materials ......................................................................................................................... 110
  4.3.2 Release kinetics of hexanal ........................................................................................................ 110
  4.3.3 Postharvest hexanal vapour treatment of bell peppers ......................................................... 111
  4.3.4 Analysis of volatile compounds of pepper fruit exposed to hexanal vapour ........................... 111
  4.3.5 Statistical analysis .................................................................................................................... 112
4.4 Results and discussion .................................................................................................................. 112
  4.4.1 Release of kinetics of hexanal .................................................................................................. 112
  4.4.2 Effect of hexanal treatment time on quality parameters of pepper fruit ............................... 114
    4.4.2.1 Effect of treatments on fruit colour ......................................................................................... 114
    4.4.2.2 Effect of treatments on fruit firmness .................................................................................... 117
    4.4.2.3 Effect of treatments on fruit ripening, physical appearance and shelf life ............................ 118
  4.4.3 Effect of hexanal treatment on volatile compounds in bell peppers .................................... 120
4.5 Conclusion ............................................................................................................................................. 124

Chapter 5 Postharvest hexanal vapor treatment delays ripening and enhance shelf life of greenhouse grown sweet bell pepper (Capsicum annum L.) ...... 125
5.1 Abstract ............................................................................................................................................ 125
5.2 Introduction ...................................................................................................................................... 125
5.3 Materials and methods ............................................................................................................... 129
  5.3.1 Plant material .......................................................................................................................... 129
  5.3.2 Postharvest hexanal vapor treatment of bell pepper fruit .................................................... 130
5.3.3 Analysis of quality parameters .......................................................... 131
5.3.3.1 Colour measurements ................................................................. 131
5.3.3.2 Measurement of fruit firmness ...................................................... 132
5.3.3.3 Measurement of respiration .......................................................... 132
5.3.3.4 Electrical conductivity/electrolyte leakage .................................... 132
5.3.3.5 Measurement of weight loss ......................................................... 133
5.3.3.6 Evaluation of shelf life quality during storage ............................... 133
5.3.4 Extraction and analysis of antioxidant enzyme activities ................. 133
5.3.4.1 Protein extraction ......................................................................... 133
5.3.4.2 Antioxidant enzymes activity analysis .......................................... 134
5.3.4.2.1 Superoxide dismutase (SOD) .................................................... 134
5.3.4.2.2 Catalase (CAT) ......................................................................... 135
5.3.4.2.3 Glutathione reductase (GR) ....................................................... 135
5.3.4.2.4 Guaiacol peroxidase (POX) ....................................................... 135
5.3.4.2.5 Ascorbate peroxidase (APX) ...................................................... 136
5.3.5 Statistical analysis ............................................................................ 136
5.4 Results ................................................................................................. 137
5.4.1 Laboratory-scale experiment .............................................................. 137
5.4.1.1 Effect of hexal vapour treatments on quality parameters .......... 137
5.4.1.1.1 Effect of hexal vapour treatments on ripening, physical appearance and shelf life ........................................ 137
5.4.1.1.2 Effect of hexal vapour treatments on fruit colour ................. 138
5.4.1.1.3 Effect of hexal vapour treatments on fruit firmness ............... 140
5.4.1.1.4 Effect of hexal vapour treatments on electrical conductivity .. 141
5.4.1.1.5 Effect of hexal vapour treatments on physiological Weight loss .................................................................. 142
5.4.1.1.6 Effect of hexal vapour treatments on fruit respiration rate ..... 143
5.4.1.2 Effect of hexal treatments on protein content and antioxidant enzyme activities .............................................. 144
5.4.1.2.1 Effect of hexal vapour treatments on protein concentration levels .................................................................. 144
5.4.1.2.2 Effect of hexal vapour treatments on superoxide dismutase (SOD) activity .................................................. 145
# LIST OF TABLES

Table 3.1. Effect of pre-harvest spraying of aqueous formulations of hexanal and EFF on colour parameters of tomato fruit ........................................... 91

Table 3.2. Effect of pre-harvest spray applications of aqueous formulations of hexanal and EFF on citric acid levels of homogenates of tomato fruit ..... 95

Table 3.3. Effect of pre-harvest spray applications of aqueous formulations of hexanal and EFF on average fruit weight of tomatoes ......................... 95

Table 3.4. Effect of pre-harvest spray applications of aqueous formulations of hexanal and EFF on average yield of tomatoes ...................................... 96

Table 3.5. Effect of postharvest dip treatment of tomato fruit in aqueous formulation of hexanal and EFF on colour parameters of tomato fruit ......................... 97

Table 3.6. Effect of postharvest dip treatment of tomato fruit in aqueous formulation of hexanal and EFF on quality parameters of tomato fruit ......................... 99

Table 4.1. Aroma substances in fruits and a description of their odour .................. 108

Table 4.2. Effect of hexanal vapour treatments on firmness and colour parameters of pepper fruits ................................................................. 115

Table 4.3. Volatile compounds of untreated bell peppers .................................. 122

Table 4.4. Volatile compounds of bell peppers treated with hexanal vapour .......... 123

Table 5.1. Effect of hexanal vapour treatments on colour coordinates of pepper fruit ................................................................. 139

Table 5.2. Commercial scale trial to study the effect of hexanal vapour treatment on colour coordinates of pepper fruit .................................................. 153

Table 5.3. Commercial scale trial to study the effect of hexanal vapour treatment on quality parameters of pepper fruit ................................. 153
LISTS OF FIGURES

Figure 2.1. The climacteric pattern of respiration in ripening fruit ......................... 35
Figure 2.2. Ethylene biosynthesis pathway .............................................................. 44
Figure 2.3. Effect of adding and removing ethylene from the atmosphere surrounding tissues that respond with a +ve or –ve feedback .................. 45
Figure 2.4. Interactions among a plant and ethylene in its environment ................. 46
Figure 2.5. Pepper fruit shiveled, caused by water loss ........................................ 47
Figure 2.6. Structure of phospholipids ..................................................................... 58
Figure 2.7. Chemical structure of phosphatidic acid ............................................... 59
Figure 2.8. Different phospholipases and their sites of action on a phospholipid ..... 62
Figure 2.9. Phospholipids and phospholipases ......................................................... 64
Figure 2.10. Schematic representation of various reactions involved in membrane deterioration .................................................................................. 65
Figure 2.11. Lipoxygenase pathway in fruits ......................................................... 75
Figure 3.1. Tomatoes harvested after pre-harvest spray applications of hexanal and EFF ............................................................................................................. 85
Figure 3.2. Effect of pre-harvest spraying of aqueous formulations of hexanal and EFF on tomato fruit firmness ................................................................. 92
Figure 3.3. Effect of pre-harvest spraying of aqueous formulations of hexanal and EFF on soluble solids content of homogenates of tomato fruit .......... 93
Figure 3.4. Effect of pre-harvest spraying of aqueous formulations of hexanal and EFF on ascorbic acid levels of tomato fruit ........................................ 94
Figure 3.5. Effect of postharvest dip treatment of hexanal and EFF on tomato fruit shelf life ........................................................................................................ 100
Figure 4.1. Effect of temperature on the concentration of hexanal in the headspace of bell pepper and tomato fruits ................................................................. 109
Figure 4.2. Pattern of hexanal release without pepper fruit during 3.5 h .............. 113
Figure 4.3. Pattern of hexanal release in presence of pepper fruit during 3.5 h ....... 114
Figure 4.4. Firmness of pepper fruit subjected to 0.01% (w/w) post-harvest hexanal vapour treatments for 0 – 16 h ................................................................. 118
Figure 4.5. Appearance of pepper fruit during storage, subjected to 0.01% (w/w) post-harvest hexanal vapour treatments for 0 – 16 h ........................................ 119
Figure 4.6. GC-MS chromatogram of volatile compounds of untreated and hexanal treated bell pepper fruit ................................................................. 121
Figure 5.1. Hexanal vapour treatment of bell pepper fruit ..................................... 131
Figure 5.2. Appearance of pepper fruit during storage subjected to different
post-harvest hexanal vapour treatments .................................................. 138

Figure 5.3. Effect of hexanal vapour treatments on firmness of pepper fruit stored for 21 d ........................................................................................................ 140

Figure 5.4. Effect of hexanal vapour treatments on electrical conductivity of pepper fruit stored for 21 d ........................................................................................................ 142

Figure 5.5. Effect of hexanal vapour treatments on weight loss of pepper fruit stored for 21 d ........................................................................................................ 143

Figure 5.6. Effect of hexanal vapour treatments on carbon dioxide production of pepper fruit stored for 21 d ........................................................................................................ 144

Figure 5.7. Effect of hexanal vapour treatments on protein concentration of pepper fruit stored for 21 d ........................................................................................................ 145

Figure 5.8. Effect of hexanal vapour treatments on SOD activities of pepper fruit stored for 21 d ........................................................................................................ 146

Figure 5.9. Effect of hexanal vapour treatments on CAT activities of pepper fruit stored for 21 d ........................................................................................................ 147

Figure 5.10. Effect of hexanal vapour treatments on GR activities of pepper fruit stored for 21 d ........................................................................................................ 148

Figure 5.11. Effect of hexanal vapour treatments on POX activities of pepper fruit stored for 21 d ........................................................................................................ 149

Figure 5.12. Effect of hexanal vapour treatments on APX activities of pepper fruit stored for 21 d ........................................................................................................ 150

Figure 5.13. Commercial scale trial to study the effect of 0.01% hexanal vapour treatment on appearance of pepper fruit during storage ............................. 152
LIST OF ABBREVIATIONS

AAT  Alcohol acetyl transferase
ACC  1-aminocyclopropene-1-carboxylate
ADH  Alcohol dehydrogenase
APX  Ascorbate peroxidase
ATP  Adenosine triphosphate
β-GAL β-galactosidase
BSA  Bovine serum albumin
CAT  Catalase
CA   Controlled Atmosphere
cv.  Cultivar
DAG  Diacylglycerols
DGK  Diacylglycerol kinase
DMSO Dimethyl sulfoxide
DPA  Days post-anthesis
EDTA Ethylenediamine tetraacetate
EFF  Enhanced freshness formulation
FAO  Food and Agriculture Organization
FW  Fresh weight
GR  Glutathione reductase
GRAS Generally regarded as safe
HPL  Hydroperoxide lyase
LAH  Lipolytic acyl hydrolase
LOOH Lipoprotein lipid hydroperoxides
LOX  Lipoxygenase
1-MCP 1-Methylcyclopropene
NADH Nicotinamide adenine dinucleotide
NIST National Institute of Standards and Technology
PA  Phosphatidic acid
PC  Phosphatidylcholine
PE  Phosphatidylethanolamine
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>PG</td>
<td>Polygalacturonase</td>
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<tr>
<td>PIP&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Phosphatidylinositol 4,5-bisphosphate</td>
</tr>
<tr>
<td>PLD</td>
<td>Phospholipase D</td>
</tr>
<tr>
<td>PME</td>
<td>Pectin methyl esterase</td>
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<tr>
<td>PMSF</td>
<td>Phenylmethylsulfonyl fluoride</td>
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<tr>
<td>POX</td>
<td>Guaiacol peroxidase</td>
</tr>
<tr>
<td>PS</td>
<td>Phosphatidylserine</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid phase microextraction</td>
</tr>
<tr>
<td>TA</td>
<td>Titratable acidity</td>
</tr>
<tr>
<td>VPD</td>
<td>Vapor pressure deficit</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
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CHAPTER 1
INTRODUCTION

Recently, the Food and Agriculture Organization of the United Nations (FAO) predicted that the world population would top eight billion by the year 2030. Therefore, the demand for food would increase dramatically. As stated in the FAO report (2013), “Agriculture: Towards 2015/30”, remarkable progress has been made over the last three decades towards feeding the world. While global population has increased over 70 percent, per capita food consumption is almost 20 percent higher. In developing countries, despite a doubling of population, the proportion of those living in chronic states of under nourishment was cut in half, falling to 18 percent in 1995/97. According to the report, crop output is projected to be 70 percent higher in 2030 than current output. Fruits and vegetables will play an important role in providing essential vitamins, minerals, and dietary fibre to the world; feeding populations in both developed and developing countries.

Fruits and vegetables are major components of a healthy diet, and have gained significant attention among consumers, especially after several epidemiological surveys (Dauchet et al., 2006; He et al., 2007) have demonstrated that increased intake of fruits and vegetables has an inverse correlation with the risk of acquiring several chronic diseases. They are major sources of essential nutrients such as vitamin A, vitamin C and folate. Well balanced diets, rich in fruits and vegetables are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies, and are also reported to reduce the risk of several diseases (Kalia and Gupta, 2006). In addition, they are rich in antioxidants such as anthocyanins, carotenoids and polyphenols. Increased intake of vitamins, antioxidants, minerals, dietary fiber (Gil et al., 2006), carotenoids, flavonoids, and other phenolics are recognized benefits from a higher consumption of fruits and vegetables. These fresh
produces protect overall health, namely by improving the immune response, helping maintain eyesight, lowering the incidence of certain types of cancer and the risk of heart disease, and preventing degenerative diseases (Kaur and Kapoor, 2001). Consumers have become more aware of fruit and vegetable quality (Batt, 2006; Hewett, 2006) and, therefore, they demand a high level of quality in the products they buy. Quality of a fresh product includes attributes and properties such as colour, texture, flavour and health promoting compounds, and possible damage or defects (Abbott, 1999). Health focused organizations typically recommend that at least five servings of fruit and vegetables are to be consumed every day as part of a balanced diet; conversely many consumers generally do not regularly eat this quantity of fresh produce even if the average consumer has access to a broad range of fresh fruit products throughout the year (Butelli et al., 2008).

A major deterrent to the availability of fresh produce is the tremendous level of wastage experienced during operations at multiple steps in the value chain. The loss in fruits and vegetables can account for 25-30 % in developed countries, and nearly 50% in underdeveloped countries (Gustavsson et al., 2011). Wastage by consumers is far higher in developed and industrialized regions of the world (Gustavsson et al, 2011. FAO, global food losses and food waste 2011). The highly perishable nature of fruit and vegetables often accounts for this postharvest waste owing to their short shelf life. More effort and attention have to be devoted to improve and optimize quality upon delivery to the consumer. Quality has to be maintained or even enhanced during storage and marketing, and it has to be considered as a central trait in produce chain management. In order to understand quality of highly perishable agricultural products, and to satisfy consumer demands, the development and maintenance of quality aspects during stages of growth, harvest and postharvest storage period have to be studied. The subjects covered in this thesis focus on pre- and post-harvest applications of hexanal formulations that affect the quality and shelf life of
tomato (*Solanum lycopersicum* L.), and sweet bell pepper (*Capsicum annuum* L.) produced in commercial greenhouses.

Greenhouse tomato and sweet bell pepper are important vegetable crops in the Canadian horticultural industry. In dollar value, tomato is the second largest vegetable crop in North America and in many other parts of the world (Thakur et al., 1996). Tomato and bell pepper comprise approximately 598 and 520 ha of area in Canadian greenhouse industry respectively, with annual production of about 304,338 and 150,000 tons, having market values worth ~ $545 and $426 million respectively (AAFC, 2017). Tomato fruits are extremely beneficial to human health due to their richness in folate, potassium, vitamin C, carotenoids and flavonoids (Rao, 2007). Many of the carotenoids, such as lycopene and β-carotene, and flavonoids help protect the consumer from developing various cardiovascular diseases and different types of cancer (Basu & Imrhan, 2006). Tomatoes are the primary source of lycopene in many people’s diets (Slimestad and Verheul, 2005). Lycopene is also responsible for the red colour present in the tomato (Sabio et al., 2003). Tomato taste is attributed to various organic acids and sugars (Slimestad and Verheul, 2005). Sweet Bell pepper is a popular vegetable consumed fresh or in a variety of processed products, both as a food and as a flavoring for other foods. Fruits are commonly used in diets because of their typical colour, pungency, taste and aroma. Fresh peppers are highly demanded by North American markets all year round. From the nutritional point of view, peppers are generally considered as a good source of most essential nutrients, being rich in antioxidants and the vitamins C, E, A and B complex (Howard et al., 2000; Palma et al., 2009). Pepper fruits are also a good source of provitamin A and oxygenated carotenoids, important for the prevention of macular degeneration and contracts (Seddon et al., 1994), and phytochemicals as flavonoids, that may reduce the risk of degenerative disease (Knekt et al., 1996). Sweet bell peppers are known to possess antimicrobial activity (Wahba et al.,
2010) and may reduce the risk of life-style related diseases such as arthritis, diabetes and cardiovascular diseases (Lee et al., 1995; Eleyinmi et al., 2002; Marin et al., 2004; Nishino et al., 2009; Ozgur et al., 2011).

Tomato and pepper fruits are harvested at a horticulturally mature stage when their nutritional qualities are optimal. The fruits of bell pepper and tomato are highly perishable and need appropriate handling and adequate care to maintain the shelf-life and quality. Tomatoes and peppers grown in the greenhouses are harvested by hand and considerable damage can occur during harvesting and handling if care is not taken to minimize scuffing and impact. However, after harvest and during transportation and storage, fruits can lose their quality and become unmarketable. Untimely destruction of cellular integrity of the produce due to stress, as occurs during harvesting, packing and storage can lead to accelerated destruction of cellular structures, resulting in the loss of quality of the produce.

Post-harvest quality of produce is influenced by physiological and pathological factors. The principal physiological factors that negatively impact the fruit during transportation, storage and marketing are water loss that may cause shrinkage, shrivelling, drying (Lownds et al., 1993; Maalekuu et al., 2002); chilling injury (Hardenburg et al., 1986; Paull, 1990); and fruit softening. Fruit softening occurs due to turgor loss, starch degradation and catabolism of the cell wall (Chen et al., 2011). Cell wall softening is due to the activity of softening enzymes such as polygalacturanase (PG), pectin methyl esterase (PME), cellulase and β-galactosidase (Chuni et al., 2010). The storage life of fruits and vegetables is also limited by pathological deterioration (Ceponis et al., 1987), such as from fungal infections caused by Botrytis cinerea and Alternaria alternate (Barkai-Golan, 1981). Botrytis can grow even at the recommended storage temperatures of fruits and vegetables (Meir et al., 1995; Barkai-Golan, 2001). The bacterial decay, caused by Pseudomonas syringae and Xanthomonas campestris, can also develop during postharvest (Delahaut & Stevenson, 2004).
Ripening is a natural process that results from several physiological and biochemical changes which transform a usually non-edible fruit into one with optimal organoleptic qualities. These processes are also associated with cell wall and cell membrane degradation. Continued degradation of these structures during postharvest storage, results in a loss of compartmentalization of ions and metabolites, leading to the loss of tissue structure and ultimately homeostasis (Paliyath et al., 2008). Phospholipase D (PLD) a lipid-degradative enzyme reduces both quality and shelf life of fruit and vegetables. Phospholipase D (PLD) was first discovered in plants more than half a century ago, and was subsequently found in animals, fungi, and bacteria (Hanahan and Chaikoff, 1947). Since that initial report, numerous studies using a wide variety of plant sources have shown that PLD is widespread in the plant kingdom, and is a nearly ubiquitous enzyme (Quarles and Dawson, 1969). Over the past several years, significant advances have been made toward understanding the biochemistry and molecular biology of PLD. This enzyme has been connected with various facets of cellular processes, particularly responses to hormones and abiotic and biotic stresses. PLD catalyzes the hydrolysis of membrane phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylglycerol (PG) etc., to yield phosphatic acid (PA) and the respective head group (Kates, 1955; Galliard, 1980; Exton, 1997). Phospholipase D (PLD) in plants has originally been proposed to be important in phospholipid catabolism, initiating a lipolytic cascade in membrane deterioration during senescence and stress (Paliyath and Thompson, 1987). Membrane deterioration is also enhanced by reactive oxygen species (ROS) produced during stress conditions associated with cold storage, postharvest handling etc. (Paliyath and Droillard, 1992). Increased expression and/or activation of PLD appear to be involved in seed germination, wounding, senescence, nutrient starvation, plant-pathogen interactions, stomatal closure and water-deficit stress.
(Chapman, 1998; Munnik et al., 1998; Fan et al., 1999; Jacob et al., 1999; Frank et al., 2000; Wang and Vestrheim, 2000). A study conducted by Pinhero et al. (2003) showed that PLD activity in cherry tomatoes increased during fruit development, which peaked at the mature green and orange stages. Phospholipase D from tomatoes has been suggested to migrate from the cytosol to the membrane, and the association progressively increases with ripening leading to an increase in membrane catabolic activities. It has been shown by Whitaker (1993) that phospholipid content declines with ripening of tomato fruit as phosphatidic acid increases via phospholipase D activity. In another study Whitaker et al. (2001) suggested that increased phospholipase D activity may be involved in loss of membrane function associated with fruit ripening of greenhouse tomatoes. Mechanical damage is the main cause of loss in postharvest horticultural products (Durigan and Mattiuz, 2007), not only because the external damage renders the fruit less attractive for consumption, but also because damaged sites are preferred entry sites for pathogens and fungi. Hence, external damage creates a threat for food safety (Van Linden et al., 2008). After wounding, plants accumulate PA, and unesterified fatty acids that are released from lipids by the action of phospholipase D (Conconi et al., 1996; Ryu and Wang, 1996; Bargmann et al., 2009). Phospholipase D is activated in fruit during chilling stress by rigidification of membranes (Pinhero et al., 1998). The softening of the pericarp flesh is the major cause for texture loss in normally ripening tomatoes, which is expected to occur as a response to wound-induced increase in enzymes targeting cell walls and membranes (Frenkel and Jen, 1989). The lipid composition of greenhouse grown tomato fruit at various stages of fruit ripeness showed a large amount of phosphatidic acid content resulting from increased phospholipase D activity (Kalra and Brooks, 1973). The changes in PLD activity in tomato fruits during on-plant and post-harvest ripening could be correlated with the total phospholipid during the ripening process (Jandus et al., 1997).
The antioxidant enzymes including peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and lipoxygenase (LOX) alleviate the potential injury resulting from chilling injury in sweet bell peppers (Wang et al., 2016). The alleviation of chilling injury and maintenance of fruit quality in peppers during cold storage was accomplished through the stimulation of antioxidant enzyme activity and antioxidant gene expression, thus leading to increased protection against oxidative damage to cellular membranes. Senescence is considered to be associated with the plant's cellular defensive system, which includes antioxidant enzymes (Han et al., 2006; Xu et al., 2009). An efficient antioxidant system can delay the senescence process, even though anti-oxidative activity in fruits decreases with aging (Zheng et al., 2007). The antioxidative systems of pepper fruits seems to be involved in the response against temperature changes (Mateos et al., 2013). Under stress conditions and senescence in plants, the generation of reactive oxygen species (ROS) including superoxide radicals (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (OH) usually takes place in different cell compartments (Apel and Hirt, 2004; Bhattaccharjee, 2005; Suzuki and Mittler, 2006; Del Rio and Puppo, 2009; Mittler et al., 2010; McCarthy et al., 2011). Plants are especially susceptible to the oxidative damage produced by ROS under changing environmental conditions (Gómez et al., 1999; Mittler, 2002; Jiménez et al., 2003). The cells of higher plants contain antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) that are able to scavenge ROS, thus allowing the cell to cope with various stress conditions (Halliwell and Gutteridge, 2007; Mittler, 2002; Noctor and Foyer, 1998; Van Breusegem et al., 2008; Foyer and Noctor, 2011; Anjum et al., 2011).

For ecologically sound and equitable reduction of post-harvest losses, there is a need for PLD inhibition technology for enhancing the shelf life and quality of greenhouse
vegetables. Earlier studies have shown that many biologically active volatile compounds, including hexanal, a natural plant volatile C₆ aldehyde and a strong inhibitor of PLD (Paliyath et al., 2003; Tiwari and Paliyath, 2011), with antifungal properties have been reported to reduce postharvest losses of fruits and vegetables. Hexanal application as a preharvest spray, postharvest dip or vapour treatment offers many advantages as it does not impair fruit color and flavour development, while delaying senescence (Paliyath et al., 2003; Paliyath and Murr, 2007). Hexanal is available commercially, and has been approved as a food additive by the U.S. Food and Drug Administration, has an ORL-MAM LD50 of 3700 mg kg⁻¹ (EAFUS, 2006) and also has a GRAS (generally regarded as safe) status. Hexanal is naturally produced during lipid peroxidation through the lipoxygenase and the hydroperoxide lyases pathways (Hildebrand et al., 1988), and gives the characteristic green flavor evolved during the wounding process in vegetables such as cucumber and beans. The advantage of hexanal over other PLD inhibitors is its volatile nature, which allows it to be applied as a vapour if required. It has been observed by Sharma et al. (2010) that the pre-harvest spray application of enhanced freshness formulation (EFF) showed better color, brightness and firmness of sweet cherries compared to the control, even after 30 days of storage at 4°C. The firmness of cherries was also increased with post-harvest application of either 0.01% (w/w) hexanal vapour alone or in combination with 1 ppm 1-Methylcyclopropene (1-MCP). These treatments also resulted in higher levels of superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities in cherry fruit. Highbush blueberry fruits treated with hexanal vapour at 900 μL L⁻¹ for 24 h immediately before storage showed significant reduction in fruit decay (50-70%) in treated fruits compared with the control (Song et al., 2010). Hexanal vapour at a concentration of 18.6 μmol L⁻¹, completely ceased the hyphae growth of Penicillium expansum and Botrytis cinerea on potato dextrose agar, and on apple slices (Song et al., 1996). The effectiveness of hexanal
vapour treatment to inhibit the spore germination of *Penicillium expansum* on apple fruit was also demonstrated by Fan et al., (2006). In other studies, hexanal vapour application to plum fruits reduced the expression of PR10 proteins, as well as, the expression of phospholipase D (El-Kereamy et al., 2009). The shelf life of fresh apple slices was significantly increased when packaged under ordinary and modified atmosphere with hexanal (Lanciotti et al., 1999). Shelf life and quality of guava fruits were improved significantly with pre-harvest spray application of hexanal formulation (Gill et al., 2016). Similar results have been obtained for pre-harvest application of hexanal to strawberry fruits (Misran et al., 2015). The PLD action was also strongly inhibited in sweet corn (*Zea mays* L.) by hexanal and hexanol (Paliyath et al., 1999). The hexanal formulation increased firmness and soluble solids properties and decreased the PLD activity when applied as spray and dip treatments to cherry fruits (Paliyath and Murr, 2007). Furthermore, the effects of hexanal vapour on storage life of tomato fruits showed that a continuous application of 40-70 μL L⁻¹ effectively suppressed the incidence of grey mold, and the fruit respiration rate was increased ~50% and reddening slowed, while single dose treatment showed minimal antifungal activity (Utto et al., 2008). The fruits of longan (*Dimocarpus longan* Lour.) were exposed to hexanal vapour at 900 μL L⁻¹, as single dose, and the results showed that fruit decay was reduced significantly when stored at 5°C for 30 days (Thavong et al., 2010). Hexanal is a naturally occurring compound produced when plant tissues are disrupted. The efficacy of hexanal for shelf-life extension of fresh products was attributed to its antimicrobial properties, much similar to other aldehydes. However, hexanal also elicits cellular decay delaying effects in many fruits and vegetables (Gonzalez-Aguilar et al., 2010; Thavong et al., 2010; Utto et al., 2008). At the cellular level, hexanal has been reported to reduce the decay of plasma membrane in the cell by inhibiting phospholipase D, which is the key enzyme that is responsible for the disruption of cellular membrane (Paliyath and
Subramanian, 2008; Paliyath et al., 2003). The effect of different amounts of hexanal on phospholipase D inhibition in tomato fruits was evaluated by Tiwari and Paliyath (2011) who demonstrated PLD activity in the cytosol was inhibited over 80% with hexanal treatment. The antifungal effect of hexanal vapour treatment is dependent on concentration and treatment duration (Song et al., 2007).

To date, hexanal has been administered for phospholipase D inhibition and enhancing the shelf life and product quality with varying level of success to a variety of plants, fruits and vegetables. However, to our knowledge, there is no available scientific literature regarding the effect of hexanal formulations on retaining the quality, shelf life and antioxidant enzymes activity of the sweet bell pepper and tomato grown under controlled environment.

1.1 Hypothesis and research objectives

**Hypothesis:** The shelf life and quality of highly perishable commodities such as tomato and bell pepper are controlled through the catabolic reactions that occur in the produce during ripening. Phospholipase D (PLD) is a key enzyme that initiates membrane deterioration which ultimately leads to loss of compartmentalization and senescence. It was hypothesized that achieving PLD inhibition in tomato and bell pepper fruit by application of hexanal could improve postharvest qualities of tomato and bell pepper by extending their shelf life and quality. To test this hypothesis, the following approaches were undertaken.

**Objective I:** Evaluate changes in quality parameters including colour, firmness, soluble solids (°Brix), ascorbic acid, organic acids and shelf-life of greenhouse tomatoes. The objectives were achieved by subjecting the fruits to preharvest spray application and postharvest dip treatment with hexanal-containing formulations.
Objective II: Examine the application of hexanal as a vapour using sweet bell peppers grown under greenhouse conditions. Various aspects of hexanal vapour application, absorption and its effects on postharvest qualities of peppers were investigated.

Objective III: Analysis of antioxidant enzyme system in bell pepper fruit and its modulation by hexanal vapour treatment.

Objective IV: Analysis of postharvest qualities of bell pepper exposed to hexanal vapour in a large scale to evaluate its potential for application in fruit storage rooms of greenhouses.
CHAPTER 2
LITERATURE REVIEW

2.1 Fruits and vegetables in human health

Diet, along with lifestyle, genetic and environmental factors, is considered a major aspect affecting longevity as well as vascular disease outcome (Trichopoulos and Willett, 1996). About 50% of all cancers have been attributed to diet (Williams et al., 1999). Chronic diseases, including cancer and cardiovascular disease, are the leading cause of morbidity and mortality worldwide (WHO, 2005); and are the main causes of death in North America. In Canada, chronic diseases account for 89% of all deaths (WHO, 2005), and for more than $CAN 93 billion in direct and indirect health care costs per year (Mirolla, 2004). Dietary intake, sedentary lifestyle, body weight and cigarette smoking have long been established as modifiable risk factors for chronic disease (Ezzati et al., 2004). In Canada, fruits and vegetables form an important segment of the food guide. It has been reported that daily servings of 5 or more variety of fruits, vegetables, and their processed products have been recommended both by the US and Canadian federal agencies (Statistics Canada, 2011) to reduce the risk of acquiring these chronic diseases. Despite the issuing of public health recommendations to reduce the burden of chronic diseases, recent studies showed that 74% of Canadians consume less than the recommended daily number of servings of fruit and vegetables (Black and Billette, 2013) Fruit and vegetables are major sources of several essential nutrients that include vitamins A and C and folic acids. In addition, fruits and vegetables are rich in antioxidants such as carotenoids, polyphenols, and anthocyanins that help combat free radicals produced within the body, and the excess production of which has been related to the development of cardiovascular diseases, Alzheimer’s, macular degeneration, and cancers (Kaur and Kapoor, 2001). Fruits and vegetables are integral
components of food in all societies; however, in some parts of the world, this is limited due to agricultural collapse or sociopolitical conflicts.

More recently, several fields of research including epidemiology, human medicine and nutrition have shown that a high intake of fruits and vegetables is associated with good health and prevention of diseases (Seeram, 2008; Maynard et al., 2003; Joshipura et al., 2001). This protection has been attributed to the level of nutrients in fruits and vegetables, as well as non-nutrient molecules called 'bioactive compounds'. These have antioxidant or other biochemical effects against pathological conditions such as several forms of cancer and cardiovascular disease, as well as inhibiting DNA synthesis, inflammation and microbial activities (Paredes-Lopez et al., 2010; Kraft et al., 2008; Shukitt-Hale et al., 2009; Cho et al., 2004; Joshipura et al., 2001).

According to the World Health Organization (WHO, 2004), low fruit and vegetable consumption is one of the top ten global risk factors for mortality. Increased fruit and vegetable consumption can help protect overall health and reduce both disease risk and burden. Fruit and vegetable intake among children is of particular interest due to growing recognition of the importance of nutrition for growth, development, and prevention of chronic diseases such as cardiovascular disease and obesity (USDHHS and USDA, 2005). A number of studies have shown that childhood fruit and vegetable consumption patterns and preferences are predictive of patterns in adolescence and adulthood (Lien et al., 2001; Kvaavik et al., 2005). It has been estimated that 2.7 million lives could be saved each year through increased and adequate fruit and vegetable consumption. In addition, this increased consumption of fruits and vegetables would decrease the worldwide non-communicable disease burden by almost 2% (Lock et al., 2005). Many consumers generally do not regularly eat the quantity of fresh produce recommended by the health organizations (Butelli et al., 2008). The high cost of purchasing these products and product quality are the
major deterrents. Furthermore, more than 20% of edible fruit purchases are discarded before being eaten (FAO, global food losses and food waste 2011).

2.2 Greenhouse industry in Canada

The greenhouse industry is an important and growing segment of the Canadian agri-food industry and plays a key role in the Canadian economy. Recently, the combined sales of fruit, vegetables and flowers have exceeded $2.5 billion, putting the greenhouse industry in the same revenue range as canola and wheat, which means that the industry accounts for about 15% of the total crop farm receipts (AAFC, 2017). These figures demonstrate that the greenhouse industry is one of the major agricultural sectors contributing to the economy of Canada. The main greenhouse vegetables crops in Canada are tomatoes (598 ha), sweet peppers (520 ha), cucumbers (397 ha) and lettuce (18 ha). In the horticulture industry, Canadian greenhouse vegetable production is concentrated mainly in Ontario and British Columbia. Ontario produces 69% of greenhouse vegetable production, and B.C. produces 15%. Canadian greenhouse vegetable growers are world leaders in utilizing advanced technology in biological pest control and computerized climate control systems. The greenhouse industry has been active in Leamington, Ontario for approximately a century, beginning between the years of 1910-1920. During the 1950’s many migrants from post-World War 2 Italy settled in southwestern Ontario, and contributed towards the vegetable greenhouse industry (Papadopoulos and Gosselin, 2007). Most greenhouse vegetable growers use hydroponics for production. This method utilizes soil-less media such as rockwool or coconut fibre (coir). Rockwool is an artificial media, composed of basaltic rock, coke, and limestone. It has excellent growth properties in that it does not interact with nutrient solutions, is lightweight, and it has a large water holding capacity. Coconut fibre (coir) is an organic growing media; it is recommended for its low price and ease of disposal.
The majority of greenhouse vegetables are grown in rockwool (55%) as opposed to coir (35%). The remaining 10% are grown in other media such as Nutrient Film Technique (NFT), foam, expanded clay pellets, peat, saw dust and soil (OMAFRA, 2010).

Product quality and safety are seen as key elements on which to build the strength of the greenhouse vegetable market. Despite the occasional problems of the greenhouse vegetable industry, the long-term prospect looks promising when one considers their market potential in North America. The optimism about the future is derived from establishment of the high levels of quality and shelflife of fruit and vegetables they produce. Even though the horticulture produce sector has been expanding, the post-harvest loss of produce due to several reasons has been a constant problem. The production sector uses well defined agronomic practices to produce high quality produce, but detailed protocols are lacking for maintaining product shelf life and quality during storage. Ripening of fruits is an early stage of senescence. Vegetables, even if physiologically immature, undergo stress after harvest that ultimately leads to loss of quality. Thus, understanding the molecular mechanisms involved in the deterioration of produce is key to developing strategies for controlling the senescence process, and enhancing the shelf life and quality of the produce. More effort and attention have to be devoted to improvement and optimization of produce quality upon delivery to the consumer. Quality has to be maintained or even enhanced during storage and marketing, and it has to be considered as a central trait in fruit chain management.

2.3 Fruit quality attributes

The concept of fruit quality refers to all attributes that consumers consciously or subconsciously believe a fruit must have. However, it is necessary to expand this concept beyond the consumer to include all who participate in the fresh produce value chain. The attributes that are associated with quality vary with context. The expected quality of a
The product is generally based on consumer preference. The quality attributes can be divided into experience attributes which are determined before and during usage (flavour, ease of preparation) and credence attributes which are based on beliefs (nutritional value, production methods, food safety) (Tijskens et al., 2001). Physical and chemical attributes are used to assess external and internal quality in fresh produce. External quality attributes relate to the appearance, and for the majority of fruits and vegetables, are:

- firmness
- colour
- absence of defects
- shrivelling (water loss)

Lately, internal quality attributes, related to flavour perception and health, have gained more importance for the consumer, and hence for the whole chain. These attributes comprise:

- taste (sweet, sour)
- aroma and flavour
- juiciness
- crispness
- absence of fibrousness
- health promoting compounds (vitamins, lycopene, glucosinolates, etc.)

### 2.3.1 Appearance and colour

Appearance is the key factor and an obvious attractant to consumers in making purchases of fresh produce. It is one of the quality factors of fresh produce for consumer preference. Fruits and vegetables are expected to have near perfect visual appearance. Appearance is used throughout the fruit production chain as the primary means of judging the quality of individual units of product (Kays, 1999). The appearance of products is commonly evaluated by considering their size, shape, form, colour, freshness condition, and finally the absence of visual defects (Costa et al., 2011). Particularly, colour is one of the most important food quality attributes affecting consumer acceptance, as it influences taste, and flavour perception (Francis, 1995; Grossman and Wisenblit, 1999; Bayarri et al., 2001;
Crisosto et al., 2003; Leon et al., 2006; Nisha et al., 2011;). Colour is considered an important grading factor for most food products since it can provide basic quality information for human perception, and has close association with quality factors such as freshness, maturity, desirability, and food safety (McCaig, 2002). Classifications of colour and ripening stages have been done for years in many developed countries. Skin colour of fruits such as tomato and sweet pepper has a strong effect on consumer acceptability of product. Variation in colour readings between maximum and minimum values increase during ripening of tomatoes, and is most variable at the pink stage of maturity (Ali et al., 2004).

Many fruits and vegetables undergo colour changes as part of the ripening and senescence process. Unripe fruit is usually green and in many types of fruit, such as apple, peach and grape, the green colour becomes lighter during ripening due to the breakdown of chlorophyll. This may reveal underlying or newly synthetized yellow or red pigments (Tucker, 1993). In some cases, fruit colour is a strong indicator of eating quality and shelf-life, for example, in tomato. Tomatoes are known for their vibrant red colour, which indicates not only maturity and therefore level of desired flavour, but also relative content of the beneficial bioactive secondary metabolites such as lycopene. Tomatoes that are deep red in colour compared to those that are lighter red or pink, are usually more mature with a desirably sweet flavour and a high content of lycopene. Important colour changes occur at various stages of tomato fruit development in terms of chlorophyll (green colour), β-carotene (orange colour) and lycopene (red colour) contents. The most visible changes are associated with chlorophyll loss (green colour) and a gradual accumulation of lycopene (red colour), where plastids such as chloroplasts present in the green fruit, are transformed into chromoplasts. Transformation of chloroplasts to chromoplasts normally occurs simultaneously with other ripening changes, such as cell wall softening (Bathgate et al., 1986). Colour transformation, together with volatile production and texture decay, is one of
the most significant and evident changes of tomato fruits during ripening. Chlorophyll and carotenoids are the major metabolites responsible for the colour of tomatoes. In the early stages of development the chlorophyll imparts a green colour while when the tomato starts the ripening process, the chlorophyll is degraded and carotenoids are synthesized. As most of the pigments that account for the perceived tomato colour are found in the pericarp, efforts have been undertaken to link colour measurements with pigment content (Arias et al., 2000). Many pre-and post-harvest factors, such as light, mineral nutrients or abiotic stress, can affect fruit colour independently of other ripeness characteristics.

In bell peppers, fruit colour is due to the presence of various plant colour pigments. Some of these pigments, including anthocyanins and carotenoids, are believed to have important health benefits (Knee 2002). These pigments are contained in either chloroplasts or chromoplasts in the outer pericarp tissues of the pepper fruits (Kirk and Juniper 1966; Govindarajan 1985). The pigment content of different pepper cultivars has been shown to be quite varied (Nagle et al., 1979). The numerous shades of peppers are due to the variations in carotenoid pigments produced by bell peppers as they ripen. The change in colour is a consequence of chlorophyll disappearance, which allows the reddish/yellowish colouration due to carotenoids to become perceptible. It has been observed that the chlorophyll degradation pathway consists of three main steps involving three different enzymes: chlorophyllase, Mg-dechelatase and pheophorbide-a-oxygenase (Vicentini et al., 1995). During the ripening of pepper fruits, de novo synthesis of carotenoid pigments occurs, and some of these (capsanthin and capsorubin) are exclusive to this genus (Minguez-Mosquera and Hornero-Mendez, 1994). This process is accompanied by a sharp decrease in chlorophylls, as a consequence of the degeneration of chloroplast into chromoplast. The role of chlorophyllase during this process seems to be important: its activity is manifested in the ripening process, perhaps being a triggering or modulating factor of the de novo
biosynthesis of carotenoid pigments (Hornero-Mendez and Minguez-Mosquera, 2002). The increase in activity has been related to senescence and maturation (Terpstra and Lambers, 1983). Furthermore, the chlorophyll and carotenoids contents of pepper can vary in composition and concentration, owing to differences in genetics and maturation (Markus et al, 1999; Russo and Howard, 2002).

Pepper harvesting time is usually determined by the fruit colour required for marketing. Sweet bell peppers for the fresh market should be harvested at optimum maturity, while fruits are firm, shiny in appearance, and have a fresh green calyx and stem. Irregular shape does not detract from edible quality, but it reduces eye appeal, which may lower market acceptability. Peppers having soft, pliable, thin flesh and pale green colour (for certain varieties) are too immature for harvest. As red sweet bell peppers mature on the plant, they tend to become sweeter and change from green to “chocolate” and then to red colour. In recent years, a premium fresh market for coloured peppers has emerged, but consistent colour is difficult to produce because the fruit must remain on the plant until the desired colour has developed (Hurst, 2009).

2.3.2 Texture

Texture is another important quality related attribute, and is the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinaesthetic (Szczesniak, 2002). Texture not only drives consumer preference, but also has a significant impact on shelf life, acceptability and transportability, and may be considered as a final quality index by which the consumer decides to purchase fresh fruit and vegetables (Batu, 1998). Textural properties can be used to determine the maturity of the fruit and vegetables. Maturation of fruits is often accompanied by softening; over-mature vegetables frequently become fibrous
or tough. Texture, as well as the other principal quality factors (Bourne, 2002), has been recognized as a multi-factorial trait, being composed of several sub-traits grouped in two main categories, mechanical (firmness, hardness, stiffness, and elasticity) and acoustic (crispness and crunchiness) (Szczesniak, 2002; Costa et al. 2012). These two categories are largely distinguished by their physical nature. Textural quality and related sensory attributes, such as juiciness, turgidity, and crispness do influence human perception of flavour and future research should contribute to improved understanding of the physical and chemical changes that contribute to desirable texture and flavour of fruits and vegetables. Overall, fruit firmness is an important factor for handling of fruits and vegetables. Firmness is a widely accepted index for evaluation of fresh products quality. In almost all agricultural products, firmness is related to maturity. Generally, fruit firmness decreases gradually as they become more mature and decreases rapidly as they ripen. Texture of over ripe and damaged products will be softer than optimum mature products. Thus firmness can be used as a criterion for quality of fruit and vegetables.

In tomatoes, texture is an important quality related attribute, and is one of the critical components of the consumer’s perception of tomato fruit quality (Causse et al., 2003; Chaib et al., 2007; Sinesio et al., 2010). Tomato texture is influenced by flesh firmness and skin strength. Softening during storage, distribution and tomato ripening can be a major problem, because it may increase their susceptibility to damage. After harvest, ripening continues and tomatoes can become overripe very rapidly. This can result in quality losses and restricted shelf-life (Geeson et al., 1985). Many traits are involved in fruit texture, including sensory attributes such as flesh firmness, mealiness, meltiness, juiciness, and crispness. Understanding the key factors that influence texture and ripening-related softening of tomato fruit has been a priority from a horticultural and commercial perspective. Major changes in texture that occur during fruit ripening considerably influence post-harvest
Tomato fruit softening usually involves one of two mechanisms: weight loss with turgor loss, or as a result of enzymatic activity. Weight loss is a non-physiological process associated with postharvest dehydration resulting in loss of turgor. Fruit weight loss is affected by several pre and postharvest factors, such as harvest date and temperature (Alia-Tejacal et al., 2007). This parameter could be used to define tomato quality, due to its impact on the flesh which becomes dull and very soft when weight loss is high. Changes in firmness related to enzymatic activity are catalysed by pectin methylesterase (PME) and polygalacturonase (PG). Enzymatic pectin degradation by PME and PG occurs in two phases: firstly, pectin is partially demethylated by PME resulting in methanol production and in a lower degree of methylation of pectin and polygalacturonic acid, and secondly, the latter is depolymerised by PG. This results in short demethylated pectin chains and consequently in drastic texture changes, namely softening (Vu et al., 2004). So far, unravelling the complex nature of texture has proven to be far more challenging than initially expected, principally because texture is influenced by many factors, including cell wall structure, cuticle properties, cellular turgor and fruit morphology (Vicente et al., 2007). Cell wall degradation is the key factor that causes softening of the several fruits and vegetables. This involves the degradation of cellulose components, pectin components, or both. Cellulose is degraded by cellulase or β-1,4-glucanase. Pectin degradation involves the enzymes pectin methylesterase, polygalacturonase, and β-galactosidase. The tomato fruit cell wall is probably the best studied with respect to changes during ripening (Brummell and Harpster, 2001; Seymour et al., 1990), but until now, the precise roles of most of the polysaccharide and glycoprotein components are still not entirely understood (McCann and Rose, 2010).

The textural features of the pepper fruit are influenced by the firmness of the whole fruit and the firmness of the fruit flesh, and are related to cultivar, ripeness rate and
susceptibility to damage during harvesting (Purkayastha and Mahanta, 2011). Firmness is one of the important factors determining market quality and consumer acceptance of bell peppers. Whether consumed green or coloured, high quality bell peppers are those fruits which are firm with a fresh crisp texture having a rich, bright colour; thick-flesh; fresh, green calyx and stem (if present) and are free of shriveling, bruises, abrasions, and diseases (Ryall and Lipton, 1979; Prabha and Sethu, 1996; Bosland and Votava, 2000). Accelerated loss of firmness is considered the greatest postharvest quality loss and limits the shelf-life of fresh tissue (ElsevieValero et al., 2007).

A rapid decrease of flesh firmness during fruit ripening has been observed, and is primarily due to changes in cell wall carbohydrate metabolism that result in a decrease of certain structural components of cell wall (Bartley and Knee, 1982). Polygalacturonase, pectin methylesterase, β-galactosidase and cellulase are the major enzymes involved in fruit softening (White, 2002). To identify biochemical characteristics related to fruit softening, Cheng et al. (2008) studied five pepper cultivars with varying degrees in flesh firmness. Firmness of fruit flesh (with epidermis attached) and (without epidermis) was measured at different developmental stages: premature (15–20 days post-anthesis (DPA), stage 1), green mature (commercially ripe, 30–35 DPA, stage 2), colour turning (fruit becoming 30–40 % red, 40–50 DPA, stage 3), and red ripe (fruit totally red, 60–70 DPA, stage 4) with a pressure tester. Firmness with and without the epidermis attached changed similarly in all pepper lines during development. The biochemical characteristics measured included insoluble pectin, soluble pectin, cellulose contents, and the activities of pectin methyl-esterase (PME), polygalacturonase (PG), β-galactosidase, and cellulase. In all varieties, flesh firmness was highest at stage 3, and then decreased during development. Soluble pectin content also increased in all cultivars. Cellulose content normally decreased after stage 3, but these changes varied among varieties. With ripening PG and PME decreased
in the most firm varieties, and cellulase and β-galactosidase were the key enzymes involved in the less firm cultivars. The authors concluded that changes of fruit firmness were to some extent correlated to the soluble pectin and cellulose content during development and ripening. However, the key biochemical characteristics causing fruit firmness changes were clearly different among the pepper fruit types. Tadesse et al. (2002) measured pepper fruit firmness in different growth stages (1-11 weeks after anthesis), using an Effegi penetrometer. Fruit firmness increased with fruit size except that a slight reduction occurred in the final two harvests. Sweet pepper weight loss has coincided with decreased firmness in numerous experiments (Ilic et al., 2012; Cuadra-Crespo and del-Amor, 2010; Diaz-Perez et al., 2007; Conforti and Zinck, 2002). The study observations are confounded by either difference in cultivar, harvesting stage or time in storage, which all have potential to influence cell wall modifications that contribute to overall fruit firmness. Firmness also varied among sweet pepper varieties indicating that the thicker pericarp tissues and high skin wax could serve as a good water reservoir potentially contributing to fruit firmness (Ilic et al., 2012).

### 2.3.3 Flavour

Flavour is typically described by aroma (odour) and taste. Aroma compounds are volatile, while taste is mainly composed of sweetness, sourness, bitterness, saltiness due to various natural salts (Peters and Amerongen, 1998), and astringency related to flavonoids, alkaloids (DeRovira, 1997), tannins (Taylor, 1993) and other factors. In addition to the external appearance and texture changes in fruits, aroma and flavour are becoming key factors that determine the choice to purchase a fruit. The perception of sweetness (sugars) is one of the most important components of fruit or vegetable flavour, and is modified by sourness or acid levels and the aroma compounds (Kader, 2008). The overall taste
sensation associated with the flavour of the fruit is dependent upon the composition of individual sugars and acids and the balance between them (Knee 2002). This composition and balance can be affected by numerous factors, including cultivar, and maturity level (Bosland and Votava, 2000). Fruits and vegetables can be classified into two major groups, depending on their flavour characteristics (Stevens, 1985). The first group has a strong flavour that can be attributed to a single compound or group of related compounds. Bananas with isoamylacetate, onions with characteristic sulfide compounds, and celery with distinctive phthalides are examples of this group. The second group of fruits and vegetables includes those whose flavour is determined by a number of volatiles, none of which conveys the specific characteristic aroma. Tomatoes, muskmelons and snap beans are the examples of this group (Stevens, 1985). Since flavour quality involves perception of the tastes and aromas of many compounds, it is much more challenging to manipulate it than other quality factors.

After genotype, the second most important factor influencing flavour quality of fruits and vegetables is the maturity stage at harvest. Taste is generally better when products are harvested fully ripe, since the sugar/acid ratio and the synthesis of aromatic volatiles increase with ripening. In the evaluation of fruit and vegetable flavour, it is important to consider off-flavours as well as desirable ones. Generally, the longer the time between harvest and fruit consumption, the greater the loss of characteristic aromatic volatiles and the development of off-flavours in the fruit and vegetables (Kader et al., 1978; Pelayo-Zaldivar et al., 2005). The off-flavours may be produced through the action of enzymes such as lipoxygenases or peroxidases, which form reactive free radicals and/or hydroperoxides that may catalyze the oxidation of lipid compounds. During these reactions, the undesirable flavours described as rancid, cardboard, oxidized, or wet dog may be developed. However, it has been reported that enzyme-catalyzed reactions may also result in desirable flavours.
For instance, hydroperoxide lyase catalyzes the production of typical tomato flavours (Anthon and Barrett, 2003). The volatile organic compounds in plants are generated from both primary and secondary metabolites, and are generally low molecular weight compounds. More than 7000 flavour volatiles have been identified and catalogued from foods and beverages (Goff and Klee, 2006). Many volatiles are produced in plant tissues at specific developmental stages such as, during flowering, ripening, or maturation. Although a single fruit or vegetable synthesizes several hundred volatiles, only a small subset generates the “flavour fingerprint” that helps animals and humans recognize the products and make a selection or avoidance food choice.

The aroma compounds of the flavour can be detected in parts-per-billion concentrations and are detected by olfactory nerve endings in the nose (DeRovira, 1997). Therefore, volatile compounds are often very significant in determining the overall flavour of a product, providing the subtle aspects of flavour (Kays 1997; Knee 2002). This is particularly true of bell peppers, which are mostly used for their complex flavours (Bosland and Votava 2000). Aroma is particularly important to the quality and overall flavour of green and colored bell peppers (Govindarajan, 1986).

Taste and good aroma are important factors influencing tomato commercialization. In recent years, consumers have complained about poor flavour in tomato. They consider the new, long-shelf life cultivars less tasty than the traditional ones, and would pay higher prices for a product with better flavour quality (Baldwin et al., 2000; Ruiz et al. 2005). The lack of tomato flavour can be explained by several reasons. Traditionally, growers have made the selection of new varieties based on yield, visual characteristics, slow ripening, resistance to diseases and transportability; while neglecting sensorial characteristics such as aroma and taste (Maul et al., 2000; Ruiz et al., 2005). Immature fruit harvesting, stage of ripeness, mechanical damage, inadequate postharvest treatments, internal bruising and
storage conditions can adversely affect the flavour of ripe tomatoes (Maul et al., 1998; Jones, 1999; Moretti et al., 2002). Also, storage at lower temperatures has detrimental effects on fruit taste and aroma (Maul et al., 2000). The characteristic flavour of a fresh tomato is the result of complex interactions between organic acids, soluble sugars and over 400 volatile compounds, that are synthesized during the ripening process in the intact fruit (Baldwin et al., 2000). From the large number of volatiles present in tomato, only about 30 are considered as impact aroma compounds, since they have positive values for odour. These volatiles are derived from different biochemical pathways, such as the catabolism of lipids, amino acids, lignins and carotenoids (Sanz et al., 1997; Baldwin et al., 2000). Malundo et al. (1995) found that taste (sweetness and sourness) and acceptability of tomatoes was greatly affected by sugar and acid levels. The study also found that sugar and acid concentrations did not significantly influence descriptive ratings for fresh tomato impact. In tomato, hexanal, (E)-2-heptenal, (E,E)-2,4-decadienal, 6-methyl-5-hepten-2-one, geranylacetone, 2-isobutylthiazole, 1-nitro-2-phenylethane and geranial increased during postharvest storage at 20°C, and only methyl salicylate decreased (Krumbein et al., 2004). These authors demonstrated that the intensity of the attribute “tomato like” aroma increased during storage but so did the undesirable attribute “mouldy”, and suggested that 2-isobutylthiazole could be responsible for the “off-flavour” occurrence detected in stored tomatoes. On the contrary, the increase in geranialacetone contributed to the “tomato-like” flavour. The aroma profile can change dramatically during the post-harvest life of fresh produce, particularly in climacteric fruits in which the dominant volatile may be quite different in the unripe fruit, the ripe fruit and the over-ripe or senescing fruit (Morton and Macleod, 1990).
2.3.4 Nutritional quality and antioxidant

In fruits and vegetables, providing higher nutritional content at affordable prices is likely to increase their consumption, which would be good for producers, growers as well as for consumers. At the same time, more attention to flavour and taste quality needs to be improved. A new diet-health paradigm is evolving, which places more emphasis on the positive aspects of diet. We are in the middle of a revolution that is changing the concept of food and our way of eating (diet). This is drawn by a more health conscious consumer base with a greater appreciation of the association between an healthy diet and reduce chronic disease risks. As such, foods which confer additional health benefits beyond basic nutrition are referred to as functional foods (Kaur and Kapoor, 2001; Dillard and Bruce German, 2000; Galland, 2013; Hurst and Hurst, 2013).

The oxidative property of oxygen plays an essential role in various biological processes. When cells use oxygen to generate energy, free radicals are formed naturally as by-products of ATP (adenosine triphosphate) generation by the mitochondria. These oxidative forms of molecules are termed reactive oxygen species (ROS), or reactive nitrogen species (RNS) and are products of cellular redox reactions (Bahorun et al., 2006). These reactive species play a dual role in humans as both toxic and beneficial compounds, since they can be either harmful or helpful to the body. The major antioxidant enzymes directly involved in the neutralization of ROS and RNS are superoxide dismutase, catalase, glutathione reductase, guaiacol peroxidase and ascorbate peroxidase (Wilcox et al., 2004; Valko et al., 2005).

It has been well established that phytochemicals, such as polyphenols and carotenoids, play significant roles in the total antioxidant capacity of fruits and vegetables. Various epidemiological studies have shown that there is a considerable association between fruit and vegetable consumption and lower risk of many diseases (Block et al.,
Phytochemicals are associated with the prevention of certain chronic diseases, including cardiovascular diseases, cancer, diabetes, osteoporosis and retinal (eye) diseases, which are especially severe in Western countries. These antioxidative phytochemicals can be classified based on their mode of action: 1) Free radical scavengers that inhibit free radical formation; 2) Singlet oxygen quenchers that donate an electron or hydrogen atom; 3) Transition metal chelator that convert reactive metal ions to stable forms (Saikat and Raja, 2011; Choe and Min, 2009). Antioxidant molecules are not all equally powerful in these varied mechanisms. For example, phenolic acids are effective in trapping free radicals, but not as good at chelating metals, while flavonoids can do both efficiently i.e. scavenge free radicals and chelate metals (Choe and Min, 2009). Nutrients and phytochemicals accumulate in plants in an organ-specific manner. Besides this organ-specific distribution, the accumulation of nutrients and phytochemicals is governed by a variety of pre- and postharvest conditions. It is, therefore, important to consider that the antioxidant content of fresh tissues can be affected by maturity, agricultural practices, temperature and storage conditions. The types of stresses to which fruits and vegetables are exposed, such as high storage temperature, ultraviolet-C irradiation (especially in the southern hemisphere) (Staehelin et al., 2001), hormone treatment, among others, also affect their antioxidant capacity (Dangl et al., 2000; Wang et al., 2007; Cho et al., 2007).

Another factor that influences the phytochemical content and the antioxidant capacity of fruit and vegetables are the postharvest storage conditions. This is the point where the fruit senescence phase starts (Hodges and deLong, 2007). Senescence, a type of programmed cell death of plant organs, is defined as a genetically regulated and orderly loss of structure and function leading up to the death of cells, organs, or whole organisms (Noodén and Guiamet, 1989; Palma et al., 2006). The production of reactive oxygen
species (ROS) has been associated with senescence of both fruits and vegetables, with lipid peroxidation often among the earliest detectable senescence symptoms (Cabello et al., 2006; Hodges and Forney, 2000). As increases in plant ROS levels are also a response to stress, and stress can induce senescence. Pigment bleaching, loss of membrane integrity, cessation of photosynthesis, changes in respiration, degradation of proteins and other macromolecules, and increases in ROS levels are all symptoms of plant senescence (Hodges et al., 2004). The majority of published work to date indicates that antioxidant potential often declines during postharvest senescence, while remaining relatively unchanged during postharvest fruit ripening (as opposed to preharvest fruit ripening). This occur despite the observation, ripening-related increases in the levels of ROS-scavenging compounds such as carotenoids and anthocyanins (Hodges and deLong, 2007). Presumably, a decreased antioxidant capacity in conjunction with an increased potential to produce ROS influences the senescence-related quality decline in postharvest fruits and vegetables. The beneficial effects of tomato consumption are generally attributed to carotenoids. The two main carotenoids present in tomato are lycopene, which is the major carotenoid compound (80-90 %), giving the red colour to the fruits (Nguyen and Schwartz, 1999), and β-carotene, which is 7-10% of the total carotenoid content (Gould, 1974). Because of the presence of long-chain conjugated double bonds, lycopene has been reported to possess higher antioxidative activity than luteolin or β-carotene (Di Mascio et al., 1989). Vitamin C and various phenolic compounds are also thought to be health-promoting compounds with antioxidant properties (Frusciante et al., 2007). Tomato antioxidant content is known to depend on the cultivar (genetic factors), maturity, and both agronomic and environmental conditions present during cultivation (Martínez-Valverde et al., 2002; Dumas et al., 2003). Lycopene, the most prevalent carotenoid in tomato, is known for many beneficial health effects. Studies have supported the role of lycopene in preventing cancer
(Rao et al., 1999), osteoporosis (Sahni et al., 2009) through its antioxidant action and down-regulation of pro-inflammatory cytokines (Markovits et al., 2009). Indeed, lycopene has also been suggested to protect human against cardiovascular disease (Arab and Steck, 2000) as well. It has been reported that lycopene, a strong radical scavenging antioxidant, synergized with other lipophilic fractions of tomato, such as vitamin E, during its antioxidant actions such as inhibition of LDL oxidation (Fuhrman et al., 2000). β-Carotene is a strong antioxidant known to protect humans from photo-oxidative damages. Studies have shown that consumption of β-carotene may contribute to the inhibition of atherosclerosis and prevention of myocardial infarction (Sies and Stahl, 1998).

Ascorbic acid is relatively stable in tomatoes because of the acidic conditions found in the tissue. However, it is easily destroyed by oxidation, exposure to light, or high temperatures. Significant losses of ascorbic acid can occur during the post-harvest storage period. Reducing the temperature from room temperature (20°C) to chill (4°C) or further reduction to freezer temperatures (−18°C) decreases the rate of loss (Davey et al., 2000). Tomatoes also contain high amounts of phenolic compounds, which exhibit a strong antioxidant activity. It has been reported that the total phenolic content of tomatoes is up to 200 mg of gallic acid equivalent per 100 g as dried weight (Kahkonen et al., 1999). Tomato polyphenols, mainly phenolic acids, are present in free soluble and insoluble forms, when they are bound to the fibre. Moreover, tomato contains flavonoids, in particular rutin and naringenin. Some papers pointed out that tomato flavonoids, due to their high antioxidant power and significant biological activities, can have a substantial role in the health benefits attributed to tomato consumption (Frasciante et al., 2007).

Bell peppers are considered an extremely nutritious, and the US Food and Drug Administration (USFDA) describes them as free of fat, fat, and cholesterol, as well as being low in sodium or calories, and high in vitamins A and C. They are also an excellent source
of many vitamins, minerals, flavonoids, and phytochemicals; including vitamin C, provitamin A, vitamin E, vitamin P (citrin), thiamine (B₁), riboflavin (B₂), and niacin (B₃) (Simonne et al., 1997; Bosland and Votava 2000). These nutritional components have been shown to occur in a wide range of concentrations in peppers. Such variation is believed to be due to differences in cultivation practices, cultivar variation, maturity levels, climate, postharvest handling, and the analytical methods used (Bosland and Votava 2000).

Leja et al., (2008) investigated sweet pepper (Capsicum annuum L.) cv. Spartacus, grown in foil tunnel. They harvested the fruits at three maturity stages: green, turning and red. The contents of total phenols, total carotenoids and evolution of endogenous ethylene were determined. They found a considerable increase in carotenoids during fruit ripening. The most distinct synthesis of carotenoids was observed when fruits were converted to the full maturity stage (red colour). A sharp increase in carotenoid content monitored during three maturity stages, and at the red/yellow stage carotenoid content showed the highest concentration in the studies of 10 varieties of sweet pepper (Deepa et al., 2007). Russo and Howard, (2002) studied how growing conditions affected the carotenoids levels in pepper fruits as they matured. They observed total carotenoids in fruits of most cultivars evaluated were not affected by production location at the green stage. At the turning stage, as well as the red stage most cultivars had higher levels of total carotenoids if glasshouse grown. However, glasshouse production did not universally improve the carotenoid content, as indicated by higher levels of capsanthin observed at the red stage in fruits of field-grown pepper (Anaheim type). It is clear there is no simple conclusion that can explain the relative amounts and changes in carotenoid levels that accompany the colour changes that occur concurrently with maturation. The various cultivars exhibit variations in the evolution, distribution and chemistry of carotenoids in pepper fruits. Hornero-Mendez and Minguez-Mosquera, (2002) suggested that carotenoid formation is a normal process, likely a result of
senescence, and independent of chlorophyll catabolism.

2.4 Fruit quality deterioration

Multiple factors can lead to loss of quality in fresh produce. Some of these factors are part of the life cycle of living produce such as, over-ripening of fruit and vegetables. Others are a consequence of the act of harvesting (Valero and Serrano, 2010). Quality deterioration of harvested fruits and vegetables is the result of a combination of physiological, mechanical, microbiological and environmental factors and conditions.

2.4.1 Physiological

When a fresh fruit is harvested and severed from the mother plant, the life processes continue in a different way. The plant organ is deprived of its source of water, nutrients, and anti-senescent hormones. As such the fruit can no longer add nutrients or water, so it has to depend on its stored reserves. When these are exhausted, the fruits undergo an ageing process leading to breakdown and deterioration (Burg and Burg, 1965). It will finally become unacceptable as food, because of this natural rot. The normal physiological processes leading to deterioration are respiration, transpiration and ethylene production. Consequently, normal factors such as transpiration and respiration ultimately lead to water loss and senescence of the product (Ben-Yehoshua and Rodov, 2003).

2.4.1.1 Respiration

Respiration is a metabolic process in which stored food, especially carbohydrates within the plant cells, is utilised to provide energy for maintaining life. In freshly harvested fruits and vegetables, physiological modifications due to respiration occur and sometimes proceed at accelerated rates. The respiration rate of a product determines its transit and postharvest life, and is often a very good indicator of the perishability of a fruit product.
(Thompson, 2003). During respiration, complex substrate molecules such as starch, sugar, and organic acids are broken down through oxidation to produce simpler molecules such as CO₂ and H₂O. This process results in the release of energy (heat) to maintain cell’s metabolism, tissue and health of the commodity (Bhande et al., 2008). Heat produced during respiration is called “vital heat” and it contributes to the refrigeration load that must be considered in designing storage rooms (Saltveit, 2016). In an “aerobic respiration process” the availability of oxygen (O₂) is very essential. The overall process of aerobic respiration involves the synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Pi) with the release of CO₂ and H₂O (Eq. 1):

\[
C_6H_{12}O_6 + 6O_2 + 38 ADP + 38 P_i \rightarrow 6CO_2 + 44 H_2O + 38 ATP
\]  

(1)

Under normal circumstances, fresh produce undergoes aerobic respiration, during which oxygen and glucose is consumed while carbon dioxide, water and heat are produced (Kays, 1991) with the regeneration of ATP, which is commonly known as energy trapping device (Uchino et al., 2004). The release of heat can be damaging for the plant tissue, causing the temperature of the produce to increase, thus leading to degradation of the commodity (Becker and Fricke, 1996). In general, the rate of deterioration is inversely proportional to the respiration of the produce (Fonseca, et al., 2002). Commodities and cultivars with higher rates of respiration tend to have shorter storage life than those with lower rates of respiration (Knee, 2002). Horticultural products can be classified based on their respiration rates (Kader, 2002) ranging between very low (<5 mg CO₂ kg⁻¹ hr⁻¹) e.g. apples, dates, cranberries, dried fruits, onions and potatoes; and extremely high (>60 mg CO₂ kg⁻¹ hr⁻¹) e.g. spinach, asparagus, lettuce, broccoli and mushrooms. During respiration, the product
accelerates the use of its internal energy and water reserves leading to the losses in nutritive values and general appearance (Kader and Rolle, 2004). Respiration process is greatly affected by relative humidity (Golob et al., 2002). As the temperature falls, the relative humidity of the air increases, slowing down respiration rate of the commodity (Paull, 1999; Yahia, 2005). Thus, shelflife and quality of fruits and vegetables can be enhanced by controlling their respiration process by maintaining proper storage conditions with low temperature and high relative humidity.

Exposure of fresh fruits and vegetables to oxygen levels below their tolerance limits, results in a transition from a respiration to a fermentation state (Kader, 1995; Richardson and Kosittrakun, 1995). Fermentation is the process in which sugar is broken down to ethanol, acetaldehyde and other metabolites causing off-flavours in the products and promotes premature ageing (Richardson and Kosittrakun, 1995; Beaudry and Lakakul, 1995; Zagory and Kader, 1988; Beaudry, 1999). When the concentration of carbon dioxide gas rises in the surrounding atmosphere, it will spoil the product causing off-flavours, tissues breakdown, failure of fruits and vegetables to ripen as well as other abnormal physiological conditions (Zagory and Kader, 1988; Kader et al., 1989; Kader, 1995).

Fruit ripening physiology has been classically defined as either climacteric or non-climacteric, which differ in their pattern in both ethylene production and respiration rates. In this context, the fruits and vegetables which show a rapid rise and fall in cellular respiration rate during ripening, for example tomato, avocado, melon, mango, banana, apricot, peach and plum, among others are said to be climacteric. For these fruits, ethylene is considered as the plant hormone responsible for their ripening process. On the contrary, non-climacteric fruits such as, pepper, citrus fruits, grapes, pineapple, and strawberries, among others, show comparatively low profile and a gradual decline in their respiration pattern and
ethylene production throughout the ripening process. (Rhodes, 1980; Reid, 1992; Lelièvre et al., 1997; Giovannoni, 2001; 2004; 2007; Adams-Philips et al., 2004; Irtwange, 2006; Barry and Giovannoni, 2007).

![Diagram of climacteric pattern of respiration in ripening fruit](image)

**Fig. 2.1.** The climacteric pattern of respiration in ripening fruit (Adapted from Saltveit, 2016).

After harvesting, the respiration rate typically declines, slowly in non-climacteric fruits and storage organs, rapidly in vegetative tissues and immature fruits. The rapid decline presumably reflects depletion of respirable substrates that are typically low in such tissues. An important exception to the general decline in respiration following harvest is the rapid and sometimes dramatic rise in respiration during the ripening of climacteric fruits (Fig. 2.1). This rise, which has been the subject of intense study for many years, normally consists of four distinct phases: 1) pre-climacteric minimum, 2) climacteric rise, 3) climacteric peak, and 4) post-climacteric decline (Saltveit, 2016).
The respiration rate of the produce is influenced by several factors such as temperature, atmospheric composition, commodity and genotype, climacteric behaviour, maturity, light, physical stress, growth regulators, and pathogen attack (Ball, 1997; Bachmann and Earles, 2000; Cortbaoui, 2005). The most important postharvest factors are temperature, atmospheric composition, and physical stress. The higher the storage temperature, the higher the respiration rate will be (Ball, 1997; Bachmann and Earles, 2000).

### 2.4.1.1.1 Temperature

Temperature is the major environmental factor that affects the deterioration rate of non-chilling sensitive commodities (Kader, 2002). Inadequate temperature can be detrimental for quality of fresh produce (Concellón et al., 2007). Over the physiological range of most crops, 0 to 30°C (32 to 86°F), increased temperatures cause an exponential rise in respiration (Saltveit, 2016). Temperature has a profound effect on the rates of biological reactions, for example metabolism and respiration. For each increase of 10°C (18°F) above optimum conditions, the rate of deterioration increases 2 to 3 times (Kader, 2002). Cooling and refrigeration are important in preserving the quality of fresh fruits and vegetables and to extending their storage life. Although respiration is normally reduced at low but non-freezing temperatures, certain commodities, mainly those originating in the tropics and subtropics, exhibit abnormal respiration when their temperature falls below 10 to 12°C (Saltveit, 2016). Respiration may increase dramatically at the chilling temperatures or when the commodity is returned to non-chilling temperatures. This enhanced respiration presumably reflects the cells efforts to detoxify metabolic intermediates that accumulated during chilling, as well as to repair damage to membranes and other subcellular structures.
(Saltveit, 2016). Enhanced respiration is only one of many symptoms that signal the onset of chilling injury (Saltveit, 2016).

Exposing harvested commodity to high temperature can initiate a favorable environment for pathogens to grow and cause serious food safety issues for consumers. Furthermore, storing crops at high temperatures was demonstrated to accelerate respiration and transpiration rates, which can further deteriorate the postharvest quality of the produce. Precooling the commodity immediately after harvest can alleviate the effect of high temperature on crop losses and extend their shelf life (Cortbaoui et al., 2005; Vigneault et al., 2007). If the temperature is allowed to fluctuate beyond the desired range, the produce may experience increased water loss and condensation on the produce from the surrounding air leading to the growth of microorganism (Llamas et al., 2013; Muratore et al., 2008; Lin, 2005). Continued exposure to elevated temperature results in phytotoxic symptoms, and then complete tissue collapse. However, conditioning and heat shocks, that is, short exposure to potentially injurious temperatures can modify the tissue's responses to subsequent harmful stresses. Temperature influence not only the appearance and shelf life but also the texture, nutritional value and organoleptic characteristics such as flavour and aroma (Burden and Wills, 1989). Vitamins degradation is often associated with storage temperature. At higher temperature, nutritional value loss, such as vitamin C degradation is rapid. For example, there is negligible vitamins C loss in lemon at 13°C but a significant loss occurred at 24°C (Lange and Cameron, 1994).

Many fruits, vegetables, and ornamentals of tropical or subtropical origin are sensitive to low temperatures (Paull, 1990). These crops are injured after a period of exposure to chilling temperatures below 10 to 15°C but above their freezing points (Wang 1990; Lyons 1973). Certain horticultural crops of temperate origin are also susceptible to chilling injury (Bramlage and Meir, 1990). Those temperate crops, in general, have lower
chilling threshold temperatures of $<5^\circ$C. At these chilling temperatures, the tissues weaken because they are unable to carry on normal metabolic processes. Various physiological and biochemical alterations and cellular dysfunctions occur in chilling-sensitive species in response to chilling stress (Raison and Orr, 1990; Wang 1982; Wang and Adams, 1982). When chilling stress is prolonged, these alterations and dysfunctions will lead to the development of a variety of chilling injury symptoms such as surface lesions, internal discoloration, water-soaking of the tissue, and failure to ripen normally (Saltveit and Morris, 1990). Often, products that are chilled will still look sound when remaining in low temperatures. However, symptoms of chilling injury become evident shortly after they are moved to warmer temperatures. Fruits and vegetables that have been chilled may be particularly susceptible to decay. Weak pathogens such as Alternaria spp., which do not grow readily on healthy tissues, can attack tissues that have been weakened by low-temperature exposure (McColloch and Worthington 1952; McColloch 1962). Maturity at harvest and degree of ripeness are important factors in determining chilling sensitivity in some fruits like avocados (Kosiyachinda and Young, 1976), honeydew melons (Lipton, 1978), and tomatoes (McColloch et al., 1966).

Tomatoes are climacteric and show a pronounced increase in respiration during ripening. The intensity and duration of the climacteric varies among cultivars (Wills et al., 1998). Tomato fruit are chilling sensitive, and the recommended storage temperature varies with the maturity stage. Mature-green fruit will ripen normally at 13 to 21°C. On the other hand, ripe tomato fruit can be stored at 10°C without visible symptoms of chilling injury, though flavor and aroma are negatively affected (Maul et al., 2000). Visual symptoms of chilling injury include pitting, non-uniform ripening, and storage decays (Wills et al., 1998). Tomato fruit produce moderate amounts of ethylene: 1 to 10 μL kg$^{-1}$ h$^{-1}$ at 20°C (Abeles et al., 1992), and are sensitive to ethylene exposure; as little as 0.5 μL L$^{-1}$ ethylene is sufficient
to trigger ripening and other associated metabolic processes (Abeles et al., 1992). Tomato storage under CA may reduce development of undesirable symptoms caused by mechanical injury (Kader, 1986). However, Moretti et al., (1999) observed that CA storage did not alleviate development of internal bruising (disruption of locular gel ripening) following impacts.

In non-climacteric fruits such as pepper, pineapple, orange, strawberry and lemon, the respiration rate increases in response to ethylene treatment. The rate of \( C_2H_4 \) production in these fruits usually ranges from 0.1 to 0.5 \( \mu L \) kg\(^{-1}\) h\(^{-1}\) (Paliyath and Murr, 2008). Peppers, being non-climacteric, produce very low levels of ethylene: 0.1 and 0.2 \( \mu L \) kg\(^{-1}\) h\(^{-1}\) at 10 and 20\(^{\circ}\)C, respectively. The use of ethylene to enhance ripening or color change is not recommended because it stimulates respiration and softening more than coloration. The most effective way to color peppers is to hold partially colored fruit at 20 to 25\(^{\circ}\)C with RH >95%. Peppers derive a slight benefit from CA storage. Low O\(_2\) atmospheres retard ripening and respiration during transit and storage. Peppers are sensitive to chilling injury when stored below 7\(^{\circ}\)C (Hardenburg et al., 1986). Symptoms include surface pitting, appearance of water-soaked areas, decay especially Alternaria and Botrytis rot, discoloration of the seed cavity near the calyx, softening without water loss, and surface lesions (Hardenburg et al., 1986; Paull, 1990, Cantwell, 2000). In peppers, chilling sensitivity varies with cultivar. Ripe or full coloured peppers are less chilling sensitive than green peppers (Paull, 1990; Cantwell, 2000; Ilic et al., 2012). Symptoms of chilling injury generally do not develop until after the fruit is removed from the chilling temperature to non-chilling temperatures (Paull 1990). Bell peppers are also very sensitive to freezing injury, which occurs at temperatures at or below 0\(^{\circ}\)C (Bosland and Votava, 2000). The freezing point of bell peppers is -0.7\(^{\circ}\)C (Kays, 1997). The symptoms of freeze injury include water-soaked tissue, extreme softening of tissue, surface pitting, shriveling, and darkened stem.
and calyx which then become water-soaked. Symptoms of freeze injury, like those of chill injury, do not show up until after being returned to non-freezing temperatures. Unlike chill injury, freezing injury only increases incidences of decay if the injured peppers are held above 5°C for at least a week (Ryall and Lipton, 1979).

2.4.1.1.2 Atmospheric composition

The composition of the storage atmosphere is an important factor involved in the deterioration of perishable commodities. The exposure of fresh horticultural crops to low \(O_2\) and/or elevated \(CO_2\) atmospheres within the range tolerated by each commodity reduces their respiration and ethylene production rates. However, outside this range respiration and ethylene production rates can be stimulated, indicating a stress response (Kader, 1986). This stress can contribute to incidence of physiological disorders and increased susceptibility to decay of the produce. The deterioration of harvested produce can further be reduced by limiting the available oxygen in the storage atmosphere, usually in combination with maintaining higher carbon dioxide levels. It is generally accepted that limiting the oxygen supply reduces the respiration rate by approximately 50% at temperatures of 20ºC to 25ºC (Raghavan et al., 1996). Raised concentration of \(CO_2\) in the storage enclosed facility can also inhibit the respiration mechanism. However, some horticultural crops are sensitive to higher than ambient \(CO_2\) concentration and can develop off-odours, discolouration and other disorders (Raghavan et al., 1996). Carbon dioxide produced during the respiratory process, if allowed to accumulate, can be harmful to many products. As such peppers can be damaged by high level of carbon dioxide (Cantwell, 2006).

Adequate \(O_2\) levels are required to maintain aerobic respiration. The accurate level of \(O_2\) that reduces respiration while still permitting aerobic respiration varies among commodities. In most crops, \(O_2\) levels at around 2% to 3% produce a beneficial reduction in
the rate of respiration and other metabolic reactions. Levels of O$_2$, as low as 1% improve the storage life of some crops, for example, apples, but only when the storage temperature is optimal. At higher storage temperatures, the demand for adenosine triphosphate (ATP) may outstrip the supply and promote anaerobic respiration (Uchino et al., 2004). The need for adequate O$_2$ should be considered in the selection of postharvest handling procedures, such as waxing and other surface coatings, film wrapping, and packaging.

Respiratory metabolism of commodities depends on the storage atmospheric composition, and the kind of commodity to be stored (Kader, 2003). The shift from aerobic to anaerobic respiration depends on fruit maturity and ripeness stage (gas diffusion characteristics), temperature, and duration of exposure to stress-inducing concentrations of O$_2$ and/or CO$_2$ (Kader, 2003). Up to a point, fruits and vegetables are able to recover from the detrimental effects of low O$_2$ and high CO$_2$ stresses (fermentative metabolism) and resume normal respiratory metabolism upon transfer to air. As a result, it is essential that the carbon dioxide concentration must be maintained at a safe level through adequate ventilation or absorption of molecules (Thanh, 2006). According to Cantwell (2006), at 10ºC, high CO$_2$ (> 5%) can cause calyx discolouration, skin pitting, discolouration and softening in sweet peppers. A 3% O$_2$ + 5% CO$_2$, the atmosphere is more beneficial for sweet bell peppers stored at 5 to 10ºC for three to four weeks without appreciable chilling injury or other quality loss (Cantwell, 2006). Elevated-CO$_2$ atmospheres inhibit activity of ACC synthase (key regulatory site of ethylene biosynthesis), while ACC oxidase activity is stimulated at low CO$_2$ and inhibited at high CO$_2$ concentrations and/or low O$_2$ levels (Kader, 2003). Ethylene action is inhibited by elevated CO$_2$ atmospheres. Optimum atmospheric compositions retard chlorophyll loss (green color), biosynthesis of carotenoids (yellow and orange colors) and anthocyanins (red and blue colors), and biosynthesis and oxidation of phenolic compounds (brown color). Controlled atmospheres slow down the activity of cell
wall degrading enzymes involved in softening and enzymes involved in lignification, leading to toughening of vegetables (Kader, 2003). Low O\textsubscript{2} and/or high CO\textsubscript{2} atmospheres influence flavour by reducing loss of acidity, starch to sugar conversion, sugar interconversions, and biosynthesis of flavour volatiles (Kader, 2003). When produce is kept in an optimum atmosphere, ascorbic acid and other vitamins are retained, resulting in better nutritional quality (Kader, 1986).

2.4.1.1.3 Physical stress

Physical stress can disturb respiration, while physical abuse can cause a substantial rise in respiration that is often associated with increased ethylene evolution. The signal produced by physical stress migrates from the site of injury and induces a wide range of physiological changes in adjacent, injury free tissue. Some of the more important changes include enhanced respiration, ethylene production, phenolic metabolism, and wound healing. However, in some tissues, wounding stimulates developmental changes, such as promotion of ripening, that result in a prolonged increase in respiration. Ethylene stimulates respiration and stress-induced ethylene may have many physiological effects on commodities besides stimulating respiration. Ethylene molecules in the air bind to the receptor sites, and act like a “key” to unlock the receptors, which send a chemical signal to plant cells to perform a series of reactions (Blankenship, 2001; Choi and Huber, 2008). This chemical reaction results in ripening of fruits which is visually noticeable in certain fruits in the form of changes in colour, texture and aroma. Some composition of fruit such as water, starch and sugar content, as well as acidity of the fruit will also change. All plant cells produce low levels of ethylene; however, anything that causes stress to the plant tissues will stimulate ethylene synthesis. Stressors may include excessive water loss, physical damage or pathogenic attack. Ethylene enhances the fresh produce growth at specific stages of plant development,
including seed germination, fruit ripening, leaf senescence and abscission (Yueming et al., 1999). Exposure to exogenous ethylene can lead to an acceleration of maturation and senescence. For example, green vegetables lose their chlorophyll more rapidly, thickened fibres can develop in asparagus, premature ripening can occur in unripe fruits; cabbages and cauliflowers can lose their leaves through accelerated leaf abscission (Thompson et al., 1982; Hodges and Forney, 2000; Forney and Toivonen, 2004). Ethylene production depends on the commodity and maturity of fresh produce as well as the temperature of the environment. Usually the ethylene production rate increases with ripeness, injury incidence, disease and temperature increase (Yahia and Brecht, 2012; Barry and Giovanni, 2007). Ethylene production in plants can also be induced by a variety of external aspects such as mechanical wounding and environmental stresses, collectively called stress-induced ethylene production (Abeles et al., 1992). Excess ethylene gas production has adverse effects on plants such as causing discoloration, wilting, softening, scald and loss of crunch. Ethylene production may peak either before or after the climacteric respiration rate peak (Barry et al., 2005).

The biochemical features of the ethylene biosynthesis pathway in higher plants are well defined and have been reviewed previously (Bleecker and Kende, 2000). Plants produce C2H4 through an actively regulated biosynthetic pathway in which the amino acid methionine is used by plants in ethylene biosynthesis. The biosynthesis of the hormone starts with conversion of methionine to S-adenosyl methionine (SAM) by the enzyme methionine adenosyl transferase (Fig. 2.2). The SAM is further converted into 1-aminocyclopropene-1-carboxylate (ACC) using the enzyme ACC synthase, which determines the rate of ethylene production, thus the regulation of this enzyme during ethylene production. Finally, with the action of the enzyme ACC-oxidase (ACO) and in presence of oxygen and carbon dioxide, ethylene is produced. Ethylene biosynthesis can
be induced by exposure to endogenous or exogenous ethylene.

![Ethylene biosynthesis pathway](image)

**Fig. 2.2.** Ethylene biosynthesis pathway (Speris et al., 2001)

Each reaction in the synthesis and action of \( \text{C}_2\text{H}_4 \) involves a biological catalyst. Enzyme activity is regulated either through its synthesis and/or destruction, or by interactions with substrates and products. These interactions can create a positive or a negative feedback of \( \text{C}_2\text{H}_4 \) during synthesis (Fig. 2.3). In vegetative tissue and in non-climacteric and immature climacteric fruit tissue, \( \text{C}_2\text{H}_4 \) suppresses its own synthesis, while ripening climacteric fruit \( \text{C}_2\text{H}_4 \) enhances its own synthesis (Saltveit, 2016). This positive feedback of \( \text{C}_2\text{H}_4 \) on \( \text{C}_2\text{H}_4 \) synthesis is called autocatalytic \( \text{C}_2\text{H}_4 \) production.
Fig. 2.3. Effect of adding and removing ethylene from the atmosphere surrounding tissues that respond with a positive (ethylene promotes its own synthesis) or negative (ethylene inhibits its own synthesis) feedback (Adapted from Saltveit, 2016).

During fruit maturation, several structural and biochemical changes occur, which confer specific organoleptic qualities, such as modifications in the fruit’s external aspect, texture and flavour (Seymour, 1993). Most physical and biochemical changes that characterize the tomato ripening process are associated with alterations in the enzymatic activity. Invertase (Iki et al., 1978) and polygalacturonase (Tucker and Grierson, 1982), increase during the tomato fruits ripening, and citrate synthase and malate dehydrogenase (Jeffery et al., 1984), decrease considerably during ripening. There are some significant interactions between plant, and its environment that are important in understanding how to control biological activity of ethylene in plants (Fig. 2.4).
Fig. 2.4. Interactions among a plant and ethylene in its environment (Adapted from Saltveit, 2016).

Ethylene in the atmosphere can have a direct effect on plant tissue by raising the internal concentration to an active level. Sources of atmospheric C$_2$H$_4$ include exhaust from trucks and forklifts, pollution from industrial activity, from the burning of fuels, and biosynthesis by diseased plants or ripening fruit. In some cases, C$_2$H$_4$, whether applied as a gas or as an ethylene-releasing compound such as ethephon, is intentionally added to the plant’s environment to stimulate desirable changes. The changes can include promotion of flowering in pineapple; ripening of tomato fruit, avocado, banana, or melon; accelerating colour and fruit maturity of apple and blueberry; degreening of citrus; altering sex expression in cucurbits; defoliation and promotion of latex secretion by rubber trees (Saltveit, 1999).

2.4.1.2 Transpiration

Transpiration is a process by which plants lose their water by evaporation (Becker, et al., 1996). Moisture inside the plant tissue is moved through the outer skin, and then evaporated resulting in cooling of the commodity (Cortbaoui, 2005). At harvest, fresh fruits contain between 70 to 95 % water. Fresh produce continues to lose water after harvest, but
Unlike the growing plant, it can no longer replace the lost water from the soil. This loss of water from fresh produce after harvest is a serious problem, resulting in produce deterioration like shrinkage and loss of firmness, crispness and juiciness, reduction in nutritional quality and weight loss. Thus adversely affecting appearance, texture, flavor and mass of produce that may lead to product rejection by consumers. (Kurek et al., 2013). A high humidity level prevents water losses that may occur due to increased respiration, and lowers transpiration. When the fresh product loses 5 to 10% of its fresh weight, it begins to wilt and becomes unusable (Fig. 2.5). In order to keep water loss from fresh fruits as low as possible, produce should be kept in moist conditions. The optimum relative humidity during storage and transport is 85–95%, while the optimum relative humidity for ripening is 75–80%. Higher relative humidity will promote infection by fungi and the development of decay (Yahia, 2012).

Fig. 2.5. Pepper fruit shriveled, caused by water loss (Adapted from Cantwell, 2002).
Loss of water leading to deterioration in fruit and vegetable tissues is a common issue for postharvest handling and distribution. In addition to wilting, water stress can lead to accelerated senescence or ripening which are expressed as softening of tissues, membrane deterioration and yellowing. The speed of post-harvest water loss is dependent primarily on the external vapour pressure deficit; however, other factors will influence the situation. Products with a large surface to volume ratio such as leaf crops will lose a greater percentage of their water far quicker than large spherical fruits. The specific structure of the cuticle and the extent of suberisation in the periderm appear to be more important than thickness in improving resistance to the movement of water vapour (Gibson, 1982; Hajibagheri et al., 1983, Gibson and Nobel, 1986). Produce varies in the percentage of water which can be lost before quality is markedly reduced. Fruits with thick peels can lose a considerable amount of moisture from the skin without compromising edible quality, for example citrus species and bananas. The appearance of the fruit will, however, deteriorate steadily with increasing water loss. Other thin-skinned fruits are more susceptible to water loss, for example, table grapes (Ben-Yehoshua, 1987). Furthermore, dehydration of all products can stimulate the production of ethylene. One of the main characteristics of a fruit or vegetable that defines susceptibility to water loss is surface area to volume ratio. In beans, the density of hairs on the cuticle can modulate rates of water loss to some extent and damage to these hairs will lead to increased losses. The driving force for water loss is the vapor pressure deficit (VPD), which is the relationship that describes the difference in water activity of the fruit or vegetable and the water activity of the atmosphere surrounding it (Ben-Yehoshua and Rodov, 2003). The greater the vapor pressure deficit, the greater the water loss. Three postharvest handling principles are important in minimizing water loss of any fruit or vegetable; (1) warm product loses water faster than cool product when placed into a cool room, hence the importance of rapid precooling before storage, (2) delays in
cooling will lead to longer exposures to higher vapor pressure deficit conditions, hence timely cooling after harvest is of utmost importance, and (3) storing product at the coldest storage temperature and highest relative humidity possible will minimize water loss (Toivonen, 2011). The recommended humidity level for storage of fresh fruits and vegetables is commodity specific. For pepper, 90 to 95% relative humidity is considered to be good for storage (Cantwell, 2006) and tomato should be maintained at relative humidity levels of 85–95% (Yahia, 2012). The activities of antioxidant enzymes, such as catalase and ascorbate peroxidase, decreased under high RH (85–90%), whereas superoxide reductase increased and glutathione reductase decreased at low RH (55–60 %), an indication of their possible role in lowering rind staining in Navelina oranges (Sala and Lafuente, 2004). These observations have important implications for the fruit and vegetable industry, as harvesting may be done during extremely low RH, and the fruit may dehydrate before being stored in coolers, which are expected to be at high RH.

The time of the day of harvest may critically affect the shelf life of horticultural crops. The shelf life of sweet basil increased by almost 100 % when harvest was done late in the day compared to harvest done in the morning, a reaction that could be due partially to changes in carbohydrate composition. Accumulation of carbohydrates increases during the day as a product of photosynthesis. Increased accumulation of carbohydrates reduces CO₂ sensitivity in lettuce and chilling sensitivity in tomato seedlings (King et al., 1988; Clarkson et al., 2005). Improved shelf life due to increased extensible cell walls has been correlated to harvesting product during the late hours of the day (Clarkson et al., 2005). The same was found with leafy greens subjected to mechanical stress (Clarkson et al., 2003). Time of day of harvest has also been shown to affect the shelf life of commodities, particularly leafy greens. Shelf life was extended by as much as 6 days in arugula and 2 days in lollo rosso and red chard, when the harvest was done at the end of the day (Clarkson et al., 2005). The
time of the month or the time of the day of harvest has been associated with produce quality (Fonseca, 2009).

The rapid rate of postharvest water loss is the primary factor limiting the shelf life of bell peppers, additionally it can affect other factors influencing shelf life (Lownds et al. 1994). The maximum acceptable loss of water from bell peppers is only 7% of the original fresh weight (Kays 1997). Thus, to extend the shelf life of bell peppers, ways to slow the rate of water loss must be considered. Although the reduced temperatures of refrigerated storage help to reduce the rates of water loss, it is still important to reduce those rates even more to maintain high quality bell pepper fruits. Maintenance of a high relative humidity is essential to this task if it helps to maintain the appropriate turgor of the fruit (Yuen and Hoffman 1993; Knee 2002). There is a theory that peppers conduct the majority of their gas exchange, and thus their water loss, through the calyx (Banks and Nicholson 2000). Therefore any attempt to prevent moisture loss from pepper fruits needs to consider the calyx area of the fruit.

2.4.2 Mechanical

Mechanical damage can have a drastic effect on the postharvest quality and shelf life of most harvested fruits and vegetables. For instance, rough handling during harvesting and after harvesting can cause mechanical injuries which can affect the postharvest quality and shelf life of harvested fruits and vegetables (Arah et al., 2015). There are two types of mechanical injury that can occur during harvest and handling of fruits and vegetables; (1) cuts or punctures, and (2) impacts leading to bruising. Cuts can lead to transitory increases in respiration, ethylene production, phenolic production and cell deterioration near the site of the injury (Toivonen, 2005). Several factors influence the severity and size of bruising sustained, including maturity, water potential, tissue or cellular orientation at the site of the injury, shape of the object imparting the bruising force, energy and angle of the impact, and
temperature of the product (Miller, 2003). Cut type injuries are most prevalent in fresh-cut fruit and vegetable products. Severity of response to cutting is very much dependant on the tissue characteristics, maturity of the fruit or vegetable of interest, the coarseness or sharpness of the harvesting implement used, and the temperature at which the harvesting is done. Cut injuries occur during the harvest process of many fruits and vegetables and are more severe in machine-harvested vs hand-harvested product. Impact caused by injuries are associated with loading of product for transport, events during transport (particularly when uneven or rough roads or lanes are encountered), during unloading and throughout packaging and processing lines (Miller, 2003).

Fresh fruits need to be manipulated at different stages from the field to the industry, before reaching the consumers. At each of these stages, injuries can be induced, for instance inappropriate harvesting technique or manipulation during transport, could cause physiological and morphological changes that affect fruit and vegetables quality prior to reaching market (Shewfelt and Prussia, 1998). These injuries can be externally visible and easily detectable (skin and flesh browning and off-flavours), or not visible externally, because internal bruising is masked by external colour. However, both types of injuries result in a quality reduction (Vergano et al., 1991). Internationally, mechanical damage is the major cause of postharvest losses (FAO, 1989). The amount of admissible mechanical damage of commercialized fruits is legislated by individual countries and international organizations; an example of this legislation is provided by the European Community (CEE law 2251/92).

In mechanically injured tissues, the polygalacturonase (PG), cellulase and β-galactosidase (β-GAL) activities increased within 24 h of cutting and remained significantly higher during storage as compared to intact fruits (Karakurt and Huber, 2003). These enzyme activities were accompanied by an increase in both 1-aminocyclopropane-1-
carboxylate synthase (ACS) and 1-aminocyclopropanecarboxylate (ACC) activities raising the possibilities of enhanced ethylene production, thereby stimulating ripening (Karakurt and Huber, 2003). In tomatoes, wounding resulted in reduction or complete cessation of PG synthesis (Chung et al., 2006). The increase in PG activity during ripening is due to de novo synthesis (Tucker et al., 1980; Bird et al., 1988; Biggs and Handa, 1989), and reduction in PG gene expression was observed after wounding. Chung et al. (2006) also reported that wounding might also impair the ability of ripening tomato tissues to recover pectinesterase (PE) activity and β-galactanase activity. In tomatoes internal bruising is recognized by the appearance of yellow to green locular gel in ripe tomatoes. It is caused by an impairment of normal ripening of the locular gel following a physical impact at the green or breaker stage of ripeness (MacLeod et al., 1976). Fruit with internal bruising showed significant reductions in vitamin C content, titratable acidity (TA), and total carotenoids (Moretti et al., 1998). Besides altering quality attributes, internal bruising also affects flavor (Moretti et al., 2002). Breaker-stage tomatoes are more sensitive to internal bruising than those handled at the green stage (Sargent et al., 1992).

Bell pepper belongs to the group of non-climacteric agricultural products, which means that the changes occurring after harvest are greatly dependent on the harvest and post-harvest conditions. Bell pepper is increasingly harvested at full colour due to growing consumer demand for peppers with improved flavour and nutritional attributes (Frank et al., 2001; Fox et al., 2005). The manner in which fresh pepper is harvested directly impacts product quality and market life. The delicate nature of the skin and internal flesh should always be kept in mind while harvesting and handling the product. Wounding, bruising, and physical injury imparted to the product from rough and abusive harvesting practices will result in significant product quality loss, and an increase in postharvest decay. Damaged areas serve as entrance points to bacterial and fungal pathogens that are ever present in
the surrounding environment (Ciccarese et al., 2013; Fallik et al., 2012; Liu et al., 2007). It is a well-established fact that the highest amounts of bruise damage during harvesting occur when product pulp temperatures are at their highest (Hertog et al., 2007). The worst possible scenario for harvesting is to pick the product in the heat of the afternoon and handle it roughly. Ideally, harvesting should occur only during the cooler morning hours. It is also very important to keep the product as cool as possible after harvest, as high pulp temperatures result in accelerated rates of ripening and deterioration and reduced market value or shelf life. The harvesting carts should be placed under shade to minimize product heating in the interval between harvest and transport to the packinghouse (Nyanjage et al., 2005; David, 2004). A study of the effect of delayed cooling on field-packed peppers has shown that shelf life can be reduced by one-half if peppers are allowed to remain in full sunlight for 2 hours after harvest (Hurst, 2009).

2.5 Changes in fruits during ripening and senescence

Ripening is an important event that leads to development of organoleptic qualities in fruits (Paliyath et al., 2008). Fruit ripening is a highly coordinated, genetically programmed process occurring at the later stages of maturation and involve a series of physiological, biochemical and sensory changes; leading to the development of an edible ripe fruit with desirable quality parameters to attract seed dispersion agents (Brady, 1987; Lelièvre et al., 1997; Giovannoni, 2001). The fruit softening associated with ripening is usually represented by a decrease in the firmness of tissues involving modifications to the polysaccharide components of the primary cell wall and middle lamella that cause a weakening of the texture. It has been suggested that both the cell wall and the middle lamella must weaken for fruit to change from hard/unripe to soft/crisp and yet juicy (Brummell, 2006). The associated physiological or biochemical changes are increased rate of respiration and
ethylene production, loss of chlorophyll and continued expansion of cells and conversion of complex metabolites into simple molecules (Dhatt and Mahajan, 2007; Huber, 2008). The specific biochemical changes may vary among species, a typical change in fruits includes fruit softening due to enhanced activity of cell wall-degrading enzymes, color changes due to degradation of chlorophyll and development of carotenoids and/or flavonoids, breakdown of starch and organic acid, and modification in volatiles profile (Giovannoni, 2004; Paliyath and Murr, 2006). In some fruits and vegetables, for example, apples and tomatoes, the breakdown of intercellular adhesion between cells leads to a condition known as mealiness which is generally perceived as a loss in textural quality (Van der Valk and Donkers, 1994). In potatoes, so-called senescence sweetening is where, over time, storage starch is gradually converted to sugars. Concentrations of reducing sugars of greater than 0.1% in potato tissues being processed into chips and crisps can lead to browning or blackening of the product during the cooking process (Van der Plas, 1987). Some senescence changes can specifically affect certain types of fresh produce processing, for example, changes to the chemical and physical structure of the cell wall (Jimenez et al., 1997). Although in fresh produce, texture is highly dependent on cell turgor, the integrity of the cell wall is important to the texture of some processed products (Femenia et al., 1998). Degradation of the cell wall leads to fruit softening and weakens the first line of defense against pathogens and mechanical injuries (Heredia, 2003). However, the ultimate cause of natural senescence in cells is due to the massive degradation of membrane structure that leads to leakage of ions, metabolites and ultimately, the loss of homeostasis.

During ripening of fruit the changes that occur in the cell wall are critical to the texture of the final product. During maturation of some vegetative parts, especially stems and petioles, cell walls become lignified (Price and Floros, 1993). Lignification results in toughening of the product, such as woodiness in asparagus, broccoli, pineapple, and
rutabaga. During fruit ripening, cell wall changes include solubilization and degradation of pectin, a net loss of the non-cellulosic neutral sugars galactose and arabinose, and a possible decrease in the molecular weight distribution of hemicelluloses (Harker et al., 1997). Structural analysis of the cell wall has shown that dissolution of the middle lamellae, the pectin-rich layer between cells that provides intercellular adhesion, is the first change during ripening (Hallet et al., 1992; Jarvis et al., 2003; Yuan et al., 2006). Numerous enzymes have been suggested as being critical to these changes in the cell wall including polygalacturonases and several glycosidases, including β-galactosidase, xyl glucanase, endotransglycosylase, and cellulases (Dey and del Campillo, 1984, Huber 1992, Seymour and Gross, 1996, Harker et al., 1997). The possible role of expansins proteins that are proposed to disrupt hydrogen bonds within the cell wall has been considered (Civello et al., 1999). The use of molecular approaches, including antisense technologies, has been a powerful tool in the search for an understanding of fruit softening (Giovannoni et al., 1989). However, no single enzyme has been identified as the major determinant of fruit softening, suggesting that wall breakdown results from the coordinated action of several enzymes or that the key enzyme has not been identified.

2.6 Changes in cell membranes of the ripening fruits

Cell membranes are the highly dynamic entities that undergo constant modifications to maintain their fluidity and functionality (Paliyath et al., 2008). Lipid and protein components of the cell membrane are constantly turned over to maintain a proper functional state of the membrane suited to the physiological state of the produce. Lipids are in fact involved in several important cell functions. Besides providing the necessary amphiphilic matrix for membrane proteins and giving the cell its barrier, lipids are also involved in energy storage, cell division, biological reproduction and intracellular trafficking (Vance and
Vance, 2002). A great variety of membrane lipids have been characterized through both biochemical and biophysical studies aiming to explain differences in phase behaviour, arrangements within the bilayer and the significance this has on membrane organization (Vance and Vance, 2002; van Meer et al., 2008; Dowhan, 1997).

Membranes play a critical role in many vital activities in the cell, including intracellular compartmentalization, energy transfer, hormone binding, signal transduction, plant-pathogen interactions and transport of ions, other solutes and macromolecules between compartments. Biological membranes are thin, flexible and relatively stable sheet like structures that enclose all living cells and organelles (McKee and McKee, 2003). According to the fluid mosaic model, membranes are composed of a lipid bilayer in which integral and peripheral proteins are imbedded (Hopkins and Huner, 2004). In this fluid state the phospholipids can rotate freely, while the presence of proteins and sterols influences this fluidity (Kobiler et al., 2001). The predominance of unsaturated fatty acids also enhances the fluidity of the membrane (Leclercq et al., 2002). The biochemical composition of the lipids, the degree of unsaturation of acyl chains, polar head groups, and the pH of the medium are all factors that influence and regulate the functional properties of the membranes. The lipid composition of cell membranes can be quite heterogeneous (Yoshida and Uemura, 1986; Larsson et al., 1990).

The three major lipid classes in a eukaryotic cell are phospholipids, glycolipids and sterols with phospholipids representing the largest lipid class (Vance and Vance, 2002; van Meer and de Kroon, 2011). Among the phospholipids, the major components include phosphatidylcholine (PC), and phosphatidylethanolamine (PE), with smaller amounts of phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylserine (PS) and phosphatidic acid (PA). Most phospholipids consist of two fatty acyl chains (saturated or cis-unsaturated) which correspond to the hydrophobic part of the molecule and a glycerol
backbone carrying a hydrophilic headgroup (Vance and Vance, 2002). Figure 2.6 shows the major phospholipids in eukaryotes where the glycerol backbone carries a phosphate (phosphatidic acid) esterified to either a choline (phosphatidylcholine), ethanolamine (phosphatidylethanolamine), serine (phosphatidylserine), glycerol (phosphatidylglycerol), diphosphatidylglycerol which contains two phosphatidic acids (cardiolipin) and inositol (phosphatidylinositol).

It is the amphipathic nature of phospholipids and their ability to form bilayers that sets the physical basis for the spontaneous formation of membranes (Vance and Vance, 2002). In most eukaryotic membranes, phosphatidylcholine (PC) accounts for more than 50% of phospholipids (van Meer et al., 2008). Due to its two fatty acyl chains and a large polar head, PC has a cylindrical shape (van Meer and de Kroon, 2011). Thus PC entropy is highest when the lipid tails are turned away from water and water molecules have maximum degree of freedom (hydrophobic effect). This gives PC molecules the ability to self-assemble into a bilayer (van Meer and de Kroon, 2011).
Fig. 2.6. Structure of phospholipids. (Adapted from Vance and Vance, 2002). The chemical structure of phosphatidic acid is given in Fig. 2.7

The typical PC bears one saturated and one unsaturated fatty acyl chain. It yields a fluid (liquid crystalline) membrane with many characteristics of biomembranes. (van Meer and de Kroon, 2011). On the other hand, phosphatidylethanolamine (PE) is cone shaped due to the smaller size of its head-group (van Meer et al., 2008) and does not form lipid bilayer by itself. The non-bilayer tendency of PE is essential for the inclusion of PE in PC
bilayers and the asymmetric distribution of various lipids between the two bilayer leaflets enforce a curvature stress in biomembranes which can lead to budding, fission and fusion (van Meer et al., 2008).

During ripening and senescence, considerable changes occur that lead to alteration in the biophysical properties of membranes, including a transition from predominantly liquid crystalline to gel-phase lipid, a decrease in bulk lipid fluidity or an increase in micro viscosity, an increase in phase transition temperature, and the formation of non-bilayer lipid structures (Thompson et al., 1987). Such changes occur as a result of the enzymatic catabolism of phospholipids and the accumulation of degradation phospholipid catabolites such as phosphatidic acid (PA), diacylglycerols (DAG), free fatty acids, and their oxidation products (Paliyath and Droillard, 1992). In the past two decades, various lipid signaling molecules and particularly phosphatidic acid (PA) and phosphatidylinositol 4,5-bisphosphate (PIP$_2$) together with corresponding lipases, kinases and phosphatases, have been well documented to control cell polarity and vesicular trafficking in cells (Cazzolli et al., 2006; Lemmon, 2008). These lipids act by recruiting their effector proteins to target membranes in a specific spatiotemporal manner and/or directly affecting the biophysical properties of the membranes. PA is structurally the simplest phospholipid with just a phosphomonoster headgroup (Fig. 2.7).

![Chemical structure of phosphatidic acid](image)

**Fig. 2.7.** Chemical structure of phosphatidic acid.
Despite this simple structure, PA is important in several crucial processes of all eukaryotic cells. It serves as a key intermediate in the biosynthetic pathway of membrane phospholipids, directly affecting membrane dynamics and has important signaling functions. PA is more abundant in the plant plasma membrane, usually accounting for between 5-10% of total phospholipids (Furt et al., 2010); and can change local properties of the lipid bilayer due to its cone-shape, favoring negative membrane curvature (Kooijman et al., 2003; Testerink and Munnik, 2005). The most studied PA biosynthesis pathway involves hydrolysis of structural phospholipids by phospholipase D (PLD), directly yielding PA. In comparison to yeast and animal genomes, the PLD family is expanded in plants with 12 genes in Arabidopsis, and even more in other dicot and monocot genomes (Elias et al., 2002). PA can also be produced by phosphorylation of diacylglycerol (DAG) by diacylglycerol kinase (DGK). During senescence the bilayer undergoes certain changes such as lipid degradation that leads to impaired functioning (Thompson, 2003). When the membrane is in the liquid crystalline phase, it is very flexible and the embedded proteins can manifest maximum biological activity (Leclercq et al., 2002). When the membrane is in the gel or solid phase it is very rigid and the proteins are static, which could lead to leakages and impaired membrane functioning. Permeability changes that occur during or before the onset of ripening contribute to leakage of solutes and an increase in free space. Thompson, (2003) reported an increase in membrane permeability of fruit pulp after the respiratory climacteric. The onset of senescence is characterized by a phase change in membrane fluidity from the liquid phase to the gel phase (Leclercq et al., 2002). During senescence membranes are subjected to harmful changes viz. decline in the phospholipid content, an increase in saturated: unsaturated fatty acid ratio and a decrease in protein content. Packing defects in the boundary region between liquid crystalline and gel phase lipid domains cause the lipid bilayer to become highly permeable to ions (Thompson, 2003).
Membrane protein functioning is also affected during senescence. According to Thompson (2003) proteins are affected by the following two processes: (i) free radicals that are generated by lipid degradation attack amino acid residues and (ii) the changes in membrane fluidity induce changes in proteins by providing an unfavorable microenvironment which negatively affects ATPase activity and receptor functioning. Increases in the levels of short chain fatty acids, such as octanoic, nonanoic and decanoic acid, lead to an increase in ethylene sensitivity in petunia petals and ripening fruit (Nair and Singh, 2003). This might also increase the onset of membrane deterioration, because the increase in the short chain fatty acids to high levels could facilitate phase separation, which could lead to the formation of the gel phase (Neeta et al., 2010). Such changes affect the fluidity of the membrane and the receptor protein functioning (Pech et al., 2002).

2.7 Phospholipase D

Phospholipase D is a member of the phospholipase family of enzymes, which includes phospholipase A₁ (PLA₁), phospholipase A₂ (PLA₂) and phospholipase C (PLC). These enzymes have been classified based on their action site on the phospholipid molecule (Fig. 2.8) their regulation, function, and mode of action. PLAs cut the acyl chains from phospholipids, which produces free fatty acids and lysophospholipids. Two classes of PLCs exist, PI-PLC and nonspecific PLC (NPC), depending on their substrate specificity. Both PLCs cut the glycerol-phosphate ester bond in phospholipids to produce a phosphate containing head group and DAG. PLDs cut the head group-phosphate ester bond to produce a free head group and PA. These enzymes are present in all living organisms, are known to play important roles in remodeling biological membranes via the hydrolysis of phospholipids producing a wide array of phospholipids and their associated molecular species and molecules. Phospholipase D is associated with breakdown of the membrane.
lipid, for example PC, into choline and PA. PLD catalyzes a transphosphatidylation reaction by substituting a primary alcohol instead of water during hydrolysis, generating a substituted phosphodiester – phosphatidyl alcohol (Vance 2008).

Fig. 2.8. Different phospholipases and their sites of action on a phospholipid (Adapted from Vance, 2008).

The role of phospholipase D in the initiation of membrane deterioration during ripening and senescence has been well recognized. PLD in plants was originally proposed to be important in phospholipid catabolism, initiating a lipolytic cascade in membrane deterioration during senescence and stress (Paliyath and Droillard, 1992). Recent studies in plants indicate that PLD action plays an important role in transmembrane signaling and cellular regulation (Wang, 2002; 2005). PLD is either directly involved in signaling or mediates the signal by producing PA as secondary messenger during a wide variety of cellular and physiological process such as membrane deterioration, senescence, membrane trafficking, secretion and cytoskeleton arrangement (Rose et al., 1995; Pinhero
et al., 2003; Paliyath et al., 2008). PLD could also be involved in phospholipid turnover that maintains cell viability and homeostasis.

### 2.8 Phospholipid catabolism

The breakdown of phospholipids is an essential feature of senescence and ripening that occur in response to hormones and environmental stress (Paliyath and Droillard, 1992; Chapman, 1998; Bargmann and Munnik, 2006; Wang et al., 2006). Phospholipid catabolism is also essential to cell function and encompasses a variety of processes including metabolic channeling of unusual fatty acids, membrane reorganization and degradation, and the production of secondary messengers (Chapman, 1998). Membrane deterioration in plant senescence is commonly associated with a progressive decrease in membrane phospholipid content (Ryu and Wang, 1995). Generally, more than 50% of phospholipids are broken down during senescence along with the accumulation of their catabolic products (Paliyath and Thompson, 1987). Several classes of catalytic enzymes are responsible for the breakdown of phospholipids (Fig. 2.9) including phospholipase A (PLA), Phospholipase C (PLC) and phospholipase D (PLD). These enzymes are classified by the ester bond they cut in phospholipids. The phospholipases are not only lipid degrading enzymes, but they also participate in lipid signaling pathways, especially through the PA and DAG produced by PLD and PLC (Wang et al., 2006; Li et al., 2009; Testerink and Munnik, 2011; Okazaki and Saito, 2014; Singh et al., 2015).
Fig. 2.9. Phospholipids and phospholipases. A typical phospholipid has two acyl chains and one phosphate-containing head group attached to the glycerol backbone. Different phospholipases cut different bonds in phospholipids. Phospholipase A1 (PLA1) cuts the acyl chain at the sn-1 position, phospholipase A2 (PLA2) cuts the acyl chain at the sn-2 position, phospholipase C (PLC) cuts the ester bond between the phosphate and the glycerol, while phospholipase D (PLD) cuts the ester bond between the phosphate and the head group. (Adapted from Wang et al., 2006).

The enzyme PLD is gradually stimulated during fruit ripening in an autocatalytic manner, which can result in membrane degradation and destabilization (Paliyath and Subramanian, 2008). Phospholipase D (PLD) hydrolyses different membrane phospholipids, producing phosphatidic acid and various head groups. PLD has been characterized as having diverse roles in lipid metabolism and cellular regulation, ranging from hormone signalling (abscisic acid, jasmonate, auxin, and gibberellic acid) and environmental stress responses (drought, freezing, wounding, heavy metal toxicity, and phosphorus starvation) to involvement in various types of cellular or subcellular dynamics (Wang et al., 2006). PLD hydrolyzes glycerophospholipids at the terminal phosphodiester bond, leading to the formation of PA and a free amino alcohol group. Changes in PLD activity were observed in a number of physiological processes, including various stress injuries (Yoshida, 1979; Chetal et al., 1982; Willemot, 1983), senescence (Thompson et al., 1987), and seed aging and germination (Herman and Chrispeels, 1980; Di Nola and Mayer, 1986; Lee, 1989;
Samama and Pearce, 1993; Wang et al., 1993). It has been proposed that PLD-mediated hydrolysis is a first step in membrane deterioration in senescing carnation flowers, tomato fruits, cabbage leaves, γ-irradiated cauliflower florets, and aging cucumber and onion seeds (Paliyath et al., 1987; Thompson et al., 1987; Cheour et al., 1992; McCormac et al., 1993; Samama and Pearce, 1993; Voisine et al., 1993). The pathway for phospholipid catabolism was developed in senescing systems that involve the sequential action of enzymes such as phospholipase D (PLD), phosphatidate phosphatase, lipolytic acyl hydrolase (LAH), and lipoxygenase (Fig. 2.10).

**Fig. 2.10.** Schematic representation of various reactions involved in membrane deterioration. The autocatalytic nature of the cycle derives from the accumulation of lipid degradation products in the membrane that causes progressively increasing membrane destabilization and loss of membrane compartmentalization (adapted from Paliyath et al., 2008).

These reactions lead to the formation of oxy-free radicals and lipid peroxides that may cause membrane deterioration. PLD is the key enzyme of the pathway since none of
the following enzymes can directly act on phospholipids. Typically, phospholipid catabolism is initiated by Phospholipase D via the removal of its head group (choline), leading to the formation of phosphatidic acid (PA).

PA rarely accumulates because it is rapidly broken down to diacylglycerols (DAG) by the removal of phosphate through the action of phosphatidate phosphatase. DAG is further catabolized to free fatty acids by the action of lipolytic acyl hydrolase (LAH). LAH does not have positional specificity and can remove fatty acids from either the \( sn-1 \) or \( sn-2 \) position (Todd et al., 1990). If the fatty acids have a 1,4-pentadiene structure, they may be acted upon by lipoxygenase, yielding hydroperoxide products as well as smaller chain fatty acids and aldehydes. These unstable fatty acids hydroperoxides are further catabolized to various products and also involved in free radical generation. The free radicals may attack and impair the functionality of the membrane proteins such as ATPases and ion pumps (Thompson et al., 1987). Accumulation of such degradation products in the membrane causes destabilization through the formation of gel-phase lipid, lipid microvesicles, and non-bilayer lipid structures. The membrane compartmentalization is disrupted, resulting in leakage of calcium ions and hydrogen ions from their storage compartments such as the cell wall and the vacuole.

2.9 Phospholipase D inhibition by hexanal and enhancing shelf life

Hexanal, a six carbon aldehyde, is a naturally occurring compound produced by the oxidative degradation of fatty acids. Its presence contributes to the green flavour of many fruit and vegetables species (Croteau, 1978; Aubert and Milhet, 2007). Hexanal, an inducible, volatile, aromatic flavour compound is also produced by fruits during ripening and confers resistance to fruit after harvesting (Song et al., 1996; Lanciotti et al., 1999; Fan et al., 2006). Its volatility means that it is evaporative and the produce can be exposed to a vapour
if required. Hexanal has been approved as a food additive by the US Food and Drug Administration (FDA) (Song et al., 1996). It has lower toxicity risk to humans and animal, and causes less environmental pollution (Tripathi and Dubey, 2004). Hexanal is naturally produced during lipid peroxidation and is mediated by the lipoxygenase pathway evolved during wounding process in fruit and vegetables (Hildebrand et al., 1998). Its production increases rapidly in damaged plant tissues as a result of the activation of the lipoxygenase hydroperoxide lyase enzymatic pathway in response to wounding from herbivore or pathogen attack (Matsui, 2006).

Hexanal has antimicrobial properties and is thought to be involved in the defense mechanism of plants and in enhancing the shelf life of fruit and vegetables. In a study, the effect of a hexanal compound released from raspberries during ripening was evaluated on postharvest fungi *Colletotrichum gloeosporioides*, *Alternaria alternata* and *Botrytis cinerea*. The hexanal compound at concentration of 0.4 μL mL⁻¹, inhibited the growth of these test fungi very well (Vaughn et al., 1993). In peaches, hexanal was used to control brown rot caused by *Monilinia fructicola*, and the effect was comparable to fumigation with acetic acid (Spiers, 2001). In another similar kind of study, the effect of vapour application of hexanal was evaluated on brown rot caused by *M. fructicola* and *M. laxa* in peaches. The mycelial growth of the fungi was strongly inhibited when a single dose of 215 μL (50 μL L⁻¹ of the container) of liquid hexanal was applied at the moment of pathogen transfer (Baggio et al., 2014). Table grapes fumigated with hexanal recorded less mould growth (Archbold, 1999). A similar kind of study by Lanciotti et al., (1999) reported that hexanal totally inhibited the growth of mesophilic bacteria, yeasts and mould on apple slices. Similar trials were conducted with golden delicious apples. Hexanal vapour reduced the spore viability of *Penicillium expansum* under in vitro and in vivo conditions on apples exposed for 48 h (Fan et al., 2006). In another study, hexanal acted as an effective inhibitor of conidial germination.
of *P. expansum* which caused blue mould in pears (Neri et al., 2006). Fumigation with hexanal (900 μL L$^{-1}$ for 24 h at 20°C) significantly reduced the incidence of decay in raspberry and blueberry fruit and lesion development in peaches artificially inoculated with *M. fructicola* (Song et al., 2007). Continued exposure to hexanal (40-70 μL L$^{-1}$ for 7 days) effectively suppressed grey mould in tomato although tomato respiration increased about 50% and fruit reddening was slowed (Utto et al., 2008). According to Anusha et al. (2016), natural volatile hexanal completely inhibits the mycelial growth and spore germination of *C. gloeosporioides* and *Lasiodiplodia theobromae* on mango when applied at 0.06% (v/v) as dip treatment. Similar studies conducted by Anusuya et al., (2016), showed that pre-harvest spray of a nano-emulsion of hexanal (Enhanced Freshness Formulation) assisted in retention of mango fruits for 3-4 weeks longer on the tree while extending shelf life under storage conditions without the loss of quality of fruits.

Hexanal application also affects the gene expression profiles in various plant species. The preharvest application of hexanal induced a clear reduction in the transcript level of two phospholipase D genes and other major enzymes involved in cell wall degradation in strawberry along with improvement in shelf life of the fruits (El Kayal et al., 2017). In another study, the gene expression profiles in tomato fruits were affected by hexanal dip treatment in a similar manner to that induced by the ethylene antagonist 1-MCP (Tiwari and Paliyath, 2011). Hexanal is also considered an efficient inhibitor of PLD. The advantage of hexanal over other PLD inhibitors is its volatile nature. To evaluate the effect of hexanal on phospholipase D activity in corn kernel fractions, Paliyath et al., (1999) observed that the primary alcohol hexanol and the aldehyde hexanal were potent inhibitors of PLD activity. Hexanal was proven to be a more potent inhibitor, apparently due to the lack of a hydroxyl group that may cause interruption of the complete hydrolysis of the substrate-enzyme intermediate. These components inhibited soluble as well as membrane associated forms of
PLD (Paliyath et al., 1999). It shows that as the chain length increases, the efficacy of primary alcohols in participating in the reaction decreases, virtually showing an inhibition of the reaction in presence of water. Aldehydes such as hexanal do not possess the primary alcohol structure required for their participation in the reaction and may block the reaction at water-binding sites. Hexanal treatments showed encouraging results in enhancing the shelf life of numerous fruits such as apple, banana, cherry, peach, strawberry; broccoli, tomato, peppers, and several fresh-cut vegetables; and flowers such as carnation and rose (Paliyath and Subramanian, 2008; Cheema et al., 2014; 2018). Generally, hexanal can be applied as a vapour, spray, or dip treatment in specialized formulations containing antioxidants such as α-tocopherol and ascorbic acid (Paliyath and Subramanian, 2008). The main advantages of hexanal application over 1-MCP are that, it does not impair colour and flavour development, while delaying senescence and enhancing shelf life in fruit and vegetables (Kondo et al., 2005; Paliyath and Murr, 2007; Cliff et al., 2009; Paliyath et al., 2011). In the packaging atmosphere, hexanal considerably reduced the growth potential of the microbial population and increased colour stability of fresh sliced apples up to 16 days (Corbo et al., 2000).

2.10 Fruit volatiles

2.10.1 Volatile compounds of bell peppers

Volatile compounds are important indicators of quality in fruits (Buchanan et al., 2000). More than 125 volatile compounds have been identified in fresh and processed Capsicum fruits (Van Straten and Maarse, 1991). Although pungency is one of the most important attributes of capsicum fruits, several previous studies have analysed the volatile fraction because the chemical compounds present in this fraction directly affect the flavour (Pino et al., 2006; van Ruth et al., 2003). Moreover, the perception of the complex volatile
mixture of compounds is an important part of the consumers' selection criteria for the acceptance of food; although differences attributed to non-volatile components of food, such as sugars, alkaloids, salts and acids, also play a major role in this respect. Thus the knowledge of the composition of these volatile compounds is an important tool for differentiating between the types of capsicum, and also to establish criteria for authenticity, quality improvement, fraud prevention and assurance of origin. In addition, the food industry has an interest in obtaining concentrated aroma of peppers for flavoured products without necessarily giving pungency to food (Pino et al., 2011). The characteristic bell pepper flavor is related to volatiles such as 2-methylpropanal, 2- and 3-methylbutanal, 2,3-butanedione, 1-penten-3-one, hexanal, heptanal, β-ocimene, (E)-3-hepten-2-one, dimethyl trisulfide, and β-cyclocitral (Simian et al., 2004; Wampler and Barringer, 2012). The combination of 1-penten-3-one, (Z)-3-hexanal, (Z)-3-hexanol, 3-isobutyl-3-methoxypyrazine, and linalool is characterized as grassy, green bell pepper, and fruity notes (Simian et al., 2004). The significance of these compounds for the aroma is not yet well-known, since the research on the odour evaluation of bell pepper volatile compounds has been very limited. Buttery et al., (1969) indicated that 2-methoxy-3-isobutylpyrazine, (E,Z)-2,6-nonadienal, and (E,E)-decadienal are important aroma compounds of bell peppers because of their low threshold values and distinct odours. Some studies have described several flavour components, common to different kinds of peppers, such as, 2,3-butanedione (flavour of caramel), 1-penten-3-one (pungent, spicy), hexanal (grassy, herbal), 3-carene (red bell pepper, lemon), ocimene (sweet, herb), octanal (fruity), (E)-2-hexenal (sweet), (E)-3-hexenol, 2-sec-butyl-3-2-methoxypyrazine and isobutyl-3-methoxypyrazine (green bell pepper) (Chitwood et al., 1983; Mazida et al., 2005).

Sulfur volatile compounds are known to have contribution to the aroma of many fruits, vegetables and other food products (Wampler and Barringer, 2012). Thiols and sulfides are
the most intense, characteristic aroma compounds with sulfury, vegetable-like, fruity notes noticeable at low levels (Wampler and Barringer, 2012). However, there are only a few of these odor-active sulfur compounds in bell peppers such as methional and dimethyl trisulfide but no thiols have been reported as odorants (Wampler and Barringer, 2012). Also, 2-heptanethiol in red bell peppers is described as having a sulfury and fruity aroma (Wampler and Barringer, 2012). It was found that there are higher amounts of thiol in the cooked bell peppers than raw samples (Wampler and Barringer, 2012). Besides, higher concentrations of the thiol were found in the red of bell pepper variety compared to the green variety. It is still detected at low concentrations in raw red and green bell peppers (Simian et al., 2004). Linalool and β-damascenone present in bell peppers have a fruity aroma. The three sulfur volatiles found present in the peppers are heptane-2-thiol, 3-methyl-5-propyl-1,2-dithiolane, and 1-(2-thienyl)-pentan-1-one. These sulfur compounds at lower concentrations have been described as bell pepper, fruity, and vegetable notes. However, these same sulfur compounds in higher concentrations have sulfury, onion, and mushroom notes (Naef et al., 2008; Wampler and Barringer, 2012).

In bell peppers, water loss has been identified as the principal physiological factor limiting the quality of the pepper and its prolonged storage (Maalekuu et al., 2002). Water loss in fresh produce during the postharvest stage has been linked to several factors such as loss of membrane integrity, lipoxygenase activity and membrane lipid peroxidation. Not only does LOX activity change volatile production and pigment degradation in peppers at different ripening stages, but it can also change the membrane composition. This membrane change decreases the product quality and commercial value of the pepper. Increased LOX activity has been reported to be associated with increased water loss (Maalekuu et al., 2006). Since lipids are the major structural blocks of membranes any
change in their composition directly affects membrane stability and function. This can result in ion leakage and cellular decompartmentation (Marangoni et al., 1996).

### 2.10.2 Volatile compounds of tomatoes

Flavor is a critical attribute in the acceptance of fresh tomatoes and consumers are willing to pay a premium for full-flavored fruit due to this fact (Baldwin et al., 2000; Ties and Barringer, 2012). There have been more than 400 volatiles identified in tomato based on the results from extensive studies (Petro-Turza, 1987; Ties and Barringer, 2012). Only 29 of these 400 volatiles have been detected to be greater than 1 ppb present in tomatoes. The characteristic tomato flavor is likely to be determined by the contribution of 16 volatiles, which are (Z)-3-hexenal, β-ionone, hexanal, β-damascenone, 1-penten-3-one, 3-methylbutanal, (E)-2-hexanal, 2-isobutylthiazole, 1-nitro-2-phenylethane, (E)-2-heptanal, phenylacetaldehyde, 6-methyl-5-hepten-2-one, (Z)-3-hexanol, 2-phenylethanol, 3-methylbutanol, and methyl salicylate (Buttery, 1993; Ties and Barringer, 2012). It is also found that the aroma of a fresh, ripe tomato could be produced by combining (Z)-3-hexenal, (Z)-3-hexenol, hexanal, 1-penten-3-one, 3-methylbutanal, (E)-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and β-ionone at varying concentrations (Buttery, 1993).

The cherry tomato had nearly twice as much linolenic acid as the standard tomato (Gray et al., 1999). The linoleic/linolenic acid ratio in cherry and standard tomatoes was 1.75 and 4.1 respectively, while the hexanal/hexenal ratio on these macerated tomatoes was 0.1 and 0.27, respectively. The ratio of linoleic acid/linolenic acids was not equal to the ratio of hexanal/hexenal ((Z)-3-hexenal+(E)-2-hexenal) for cherry and standard tomatoes, indicating a simple direct correlation between the precursor fatty acids and the C₆ aroma volatiles does not exist. However, as the standard tomato had nearly twice the
linoleic/linolenic acid ratio and three times the hexanal/hexenal ratio than the cherry tomato, a crude relationship between the substrates and the products was observed (Gray et al., 1999). With addition of lipoxygenase, alcohol dehydrogenase or lipoxygenase/alcohol dehydrogenase in red tomatoes, the concentrations of hexanal, \( (Z) \)-3-hexenal, \( (E) \)-2-hexenal, and 1-penten-3-one tended to decrease instead of increase (Yilmaz et al., 2002; Ties and Barringer, 2012).

2.11 Lipoxygenase pathway in fruits

Lipoxygenase (LOX) is an enzyme found in many plants and animals, which catalyses the oxygenation of polyunsaturated fatty acids to form fatty acid hydroperoxides. Linoleic and linolenic acid are the major polyunsaturated fatty acids in plant tissues, and insertion of the oxygen takes place at either the 9 or 12 positions to generate the corresponding 9- or 13-hydroperoxides (Fig. 2.11). Lipoxygenase enzymes are present in a wide range of biological organs and tissues, but are particularly abundant in grain legume seeds (beans and peas) and potato tubers (Casey, 1998). A key role for some LOX isoforms is in the generation of fatty acid hydroperoxides destined for jasmonic acid, which triggers gene activation during wound response in plants. The fatty acid hydroperoxides generated by the activity of LOX are potentially deleterious to membrane function by causing increased rigidity and would not, therefore, be expected to accumulate (Whitaker, 2002). Lipoxygenase not only has food-related applications in bread making (Casey, 1997) and aroma production (Whitehead et al., 1995); but also has negative implications for colour, off-flavour and antioxidant status of plant based foods (Casey et al., 1996). The lipoxygenase (LOX) pathway is responsible for producing some of the volatiles which have a “green” fresh note which is desirable in many foods, but it may also cause offensive flavors in some plant systems (Ho and Chen, 1994). The main products of the LOX pathway
are C₆ volatile compounds generated through sequential enzymatic reactions. The major enzymes involved in this pathway include lipase, lipoxygenase, hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH) and (Z)-3/(E)-2 isomerase (Galliard and Matthew, 1977). The substrates of the LOX pathway are oxygen and two kinds of free fatty acids, linoleic and linolenic acid, which are released from the cell membrane by lipases following hydrolysis of phospholipids by PLD. Volatile compounds in the LOX pathway are formed during fruit ripening but their levels increase dramatically after tissue disruption (Prestage et al., 1999). In the LOX pathway, linoleic and linolenic acids are oxidized by LOX to 9- and 13-hydroperoxides. HPL then oxidizes 13-hydroperoxides to hexanal and (Z)-3-hexenal. Some (Z)-3-hexenal is isomerized to (E)-2-hexenal by (Z)-3/(E)-2 isomerase. These C₆ aldehydes can be converted to their corresponding alcohols by ADH (Stone et al., 1975).
Fig. 2.11. LOX pathway in fruits. Abbreviation: LOOH, hydroperoxide; [LOX], lipoxygenase; [HPL], hydroperoxide lyase; [ADH], alcohol dehydrogenase; [AAT], alcohol acetyl transferase; [Z3/E2 ISO], (Z)-3/(E)-2 isomerase (De Pooter and Schamp, 1989; Luning et al., 1995; Stone et al., 1975).

Several gaps in knowledge are evident based on the literature regarding the application of hexanal for phospholipase D inhibition and enhancing the shelf life and quality of vegetables grown in commercial greenhouses. Further research was specifically undertaken on evaluation of impact of hexanal applications on improving quality of greenhouse tomato, delay ripening and enhance shelf life of greenhouse
grown sweet bell peppers, volatile generation and release kinetics of hexanal. Addressing these quite divergent gaps in knowledge would help us to develop different strategies in administration of hexanal for enhancing shelf life and quality of greenhouse vegetables and ultimately benefit the greenhouse industry.
CHAPTER 3

This chapter is re-formatted from the following publication:

**Improving quality of greenhouse tomato (**Solanum lycopersicum** L.) by pre- and postharvest application of hexanal-containing formulations**

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3.1. Abstract

From harvest to consumption, tomato (**Solanum lycopersicum** L.) fruit are exposed to several exogenous factors that enhance product deterioration. Phospholipase D (PLD) is a key enzyme involved in membrane deterioration that occurs during fruit ripening and senescence. Hexanal, an inhibitor of phospholipase D has been successfully used for pre- and postharvest treatment of fruit, vegetables and flowers. In this study, effectiveness of pre- and postharvest application of an aqueous hexanal formulation and an enhanced freshness formulation (EFF) containing hexanal and other ingredients were evaluated by monitoring changes in quality parameters during postharvest storage of greenhouse tomatoes. Tomatoes subjected to preharvest spray with 1% EFF containing 1 mM hexanal twice a week had better colour, and firmness than untreated fruit and hexanal formulation treated fruit. EFF treated tomatoes also showed low hue angle values indicative of enhanced red colour. Preharvest spray with 1% (1 mM) hexanal twice a week resulted in higher levels of ascorbic acid and soluble solids in fruit than those subjected to EFF treatment, and the control. Postharvest dip application of harvested tomatoes in 2% EFF containing 2 mM hexanal resulted in enhanced brightness and hue angle values, reduced red colour, increased fruit firmness and ascorbic acid content after 21 days of storage, indicative of better quality. The results suggest that hexanal has the potential to enhance shelf-life and quality of greenhouse tomatoes.
3.2. Introduction

Greenhouse tomato (*Solanum lycopersicum* L.) is an important vegetable crop in the Canadian horticultural industry. In dollar value, tomato is the second largest vegetable crop processed in North America and in many other parts of the world (Thakur et al., 1996). According to Agriculture and Agri-Food Canada, about 322,960 tons of greenhouse tomatoes are produced annually in Canada which is worth about $509 million (Statistics Canada, 2012). Tomatoes are harvested at a mature green or vine-ripe stage, and after ripening these tomatoes show optimal nutritional qualities. However, after harvest and during transportation and storage, tomatoes can lose their quality and become unmarketable. Untimely destruction of cellular integrity of the produce due to stress, as occurs during harvesting, packing and storage can lead to accelerated destruction of cellular structures, sometimes resulting in the loss of quality of the produce. Phospholipase D (PLD) a lipid-degradative enzyme reduces both quality and shelf life of greenhouse tomatoes. Consequently, improving and preserving the quality of greenhouse tomatoes in terms of their firmness, colour, texture, flavour, and shelf life are extremely important to growers, tomato-processing companies, and the food industry in general. Postharvest losses account for nearly 40% of harvested vegetables worldwide (Gustavsson et al., 2011).

The cell membrane is a key site where initial changes associated with ripening and senescence occurs, affecting cellular compartmentalization and accelerating the senescence process (Paliyath and Subramanian, 2008; Paliyath et al., 2008). Phospholipase D (PLD), a phospholipid-degrading enzyme, catalyzes hydrolysis of membrane phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidyl- glycerol, to yield phosphatidic acid, and is the key enzyme involved in the initiation of membrane deterioration. PLD becomes associated with the membrane, initiating a lipolytic cascade of catabolic reactions leading to membrane
deterioration during senescence and stress (Paliyath and Droillard, 1992; Tiwari and Paliyath, 2011b; Paliyath et al., 2012). If the activity of PLD could be inhibited, then the rest of the enzymes are unable to act on the intermediates, which translates physiologically to enhanced membrane stability, and increasing longevity of horticultural produce. Over the past several years, significant advances have been made toward the understanding of the biochemistry and molecular biology of PLD. This enzyme has been connected with various facets of cellular processes, particularly responses to hormones and abiotic and biotic stresses. Increased expression and or activation of PLD appear to be involved in seed germination, wounding, senescence, nutrient starvation, plant-pathogen interactions, stomatal closure and water-deficit stress (Wang, 1997; Chapman, 1998; Munnik et al., 1998, Fan et al., 1999, Jacob et al.; 1999; Frank et al., 2000). A study conducted by Pinhero et al. (2003) showed that PLD activities in cherry tomatoes increased during fruit development, which peaked at the mature green and orange stages. Phospholipase D from tomatoes has been suggested to migrate from cytosol to membrane and the association progressively increases with ripening leading to an increase in membrane catabolic activities. It has been shown by Whitaker (1993) that phospholipid content declines with ripening of tomato fruit as phosphatidic acid increases via phospholipase D activity. In another study Whitaker et al. (2001) suggested that increased phospholipase D activity may be involved in loss of membrane function associated with fruit ripening of greenhouse tomatoes. In plants, PLD and its product phosphatidic acid (PA) are involved in various processes including fruit ripening, wounding and responses induced by elicitors (Meijer and Munnik, 2003). Mechanical damages are the main cause of losses in postharvest horticultural products (Durigan and Mattiuz, 2007), not only because the external damage renders the fruit less attractive for consumption, but also because damaged sites are preferred entry sites for pathogens and fungi. Hence, external damage creates a threat for food safety (Van Linden
et al., 2008). After wounding, plants accumulate PA and unesterified fatty acids that are released from lipids by the action of phospholipase D (Conconi et al., 1996; Ryu and Wang, 1996; Bargmann et al., 2009). Phospholipase D is activated in cucumber fruit during chilling stress by rigidification of membranes (Pinhero et al., 1998). The softening of the pericarp flesh is the major cause for texture loss in normally ripening tomatoes which is expected to occur as a response to wound-induced increase in enzymes targeting cell walls and membranes (Frenkel and Jen, 1989). The lipid compositions of greenhouse grown tomato fruit at various stages of fruit ripeness showed large amount of phosphatidic acid content of ripening fruits strongly suggests the result from increased phospholipase D activity (Kalra and Brooks, 1973). The changes in lipids during ripening and senescence of greenhouse grown cherry tomatoes showed higher levels of phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid. It has been suggested by Güçlü et al. (1989) that the increased levels of lipids reflected the catabolic process related to enhancement of phospholipase D activity. The changes in PLD activity in tomato fruits during on-plant and post-harvest ripening could be correlated with the total phospholipid during the ripening process (Jandus et al., 1997).

For ecologically sound and equitable minimizing of post-harvest losses, there is a need for PLD inhibition technology for enhancing the shelf life and quality of greenhouse tomatoes. A number of technologies have been developed and are currently in use to prolong shelf-life and enhance quality of fruit and vegetables. Since ethylene plays key role in fruit ripening (Carrari and Fernie, 2006; Hyang et al., 2009), some of these methods are based on functional modification of the ethylene biosynthetic path-way through inhibition of key enzymes or receptors (e.g. using aminoethoxyvinylglycine, AVG or Retain™ to inhibit ACC synthase, and exposing to 1-MCP, an ethylene receptor blocker). Though the use of 1-MCP to delay fruit ripening and softening is widely accepted for fruit such as apple and pear,
and climacteric produce in general, 1-MCP treatment can adversely affect the quality of tomato and other fruit with a soft texture. 1-MCP treatment of tomatoes can compromise flavour development, and cause incomplete suppression of colour biosynthesis resulting in blotchy ripening (Kondo et al., 2005; Lurie and Paliyath, 2008; Cliff et al., 2009; Tiwari and Paliyath, 2011a). Therefore, there is a need to find alternate technologies to delay ripening and prolong shelf-life without compromising visual, organoleptic and nutritional qualities of climacteric soft fruit such as tomato.

Previous studies have shown that activity of PLD may be selectively inhibited by primary alcohols such as hexanol and aldehydes such as hexanal (Paliyath et al., 1999; Tiwari and Paliyath, 2011a). Several technologies utilizing hexanal to prolong shelflife of produce are currently under investigation (Paliyath et al., 2003; Paliyath and Subramanian, 2008). Hexanal formulations increased fruit firmness, soluble solids, and antioxidant enzyme activity when applied as a preharvest spray (Paliyath and Murr, 2007; Sharma et al., 2010). By contrast to a pulse application as used with formulation sprays and dips, a continuous application of hexanal in the vapour form through circulating air, effectively suppressed incidence of grey mould and increased fruit respiration rate by ~50% (Utto et al., 2008). The shelf life of fresh apple slices was significantly increased when packaged under ordinary and modified atmosphere with hexanal (Lanciotti et al., 1999). It has been observed by El-kereamy et al. (2009) that hexanal reduced the expression of PLDα up regulated ABI1 and decreased PR10 expression within 24 h after treatment. Sharma et al. (2010) have observed that preharvest spray application of an enhanced freshness formulation containing hexanal and antioxidants (EFF) to sweet cherries resulted in improved colour, brightness and firmness than untreated controls and prolonged shelf-life even up to 30 days after harvest when stored at 4°C. Firmness of cherries was also
increased with postharvest application of either hexanal vapour alone, 1-MCP, or a combination of both. Fruit of longan (*Dimocarpus longan* Lour.) exposed to hexanal vapour also showed reduced fruit decay at ambient temperature storage (Thavong et al., 2010). Hexanal compositions have been administered as a preharvest spray or a postharvest dip for enhancing shelf-life and quality of a variety of fruit (e.g. apple, cherry, peach, plum, mango, guava). Previous experiments have shown that shelf-life and quality of harvested mature green tomatoes can be extended by postharvest dips in the hexanal formulation (Tiwari and Paliyath, 2011a). Effectiveness of pre- and postharvest applications of hexanal-containing formulations on enhancing shelf-life and quality of greenhouse tomatoes has not been investigated, as fruit at various growth stages are exposed to formulation. It was hypothesized that the improved effectiveness of PLD inhibition in greenhouse tomatoes was attributable to inhibitory characteristics of hexanal. Applications of hexanal composition negate the action of PLD and hence reduce the postharvest losses by enhancing the shelf life and quality of greenhouse tomatoes. Thus, the objectives of this study were to evaluate changes in quality and shelf-life of greenhouse tomatoes by preharvest spray application and postharvest dip treatment with hexanal-containing formulations.

### 3.3 Materials and methods

#### 3.3.1 Plant materials

All experiments of the study were conducted in a fully automated, commercial greenhouse belonging to Bluewater Greenhouses Ltd., situated in Vineland, Ontario, Canada (Latitude 43° 11 N, longitude 79° 24 W). Tomato plants (*Solanum lycopersicum* L.) cv. Prunus was transplanted in greenhouse and grown hydroponically in bags of rock wool with a density of 3.5 plant m⁻². The cultivation was carried out under natural light conditions using commercial production practices and standards (OMAFRA, 2010).
3.3.2 Hexanal treatment

3.3.2.1 Preharvest spray application of hexanal and EFF

Two types of formulations [hexanal and enhanced freshness formulation (EFF)] were sprayed on tomatoes at the stage of fruiting. The composition of the hexanal stock formulation was 1% (v/v) hexanal and 10% (v/v) Tween 20 dissolved in ethanol (10%, v/v). Basic ingredients of the stock formulation of EFF were 1% (v/v) hexanal, 1% (v/v) geraniol, 1% (w/v) α-tocopherol, 1% (w/v) ascorbic acid and 10% (v/v) Tween 20 dissolved in ethanol (10%, v/v). Prior to spraying, stock solutions were diluted with water and mixed properly making 1% aqueous solution to provide a hexanal concentration of 0.01% (v/v) in the final spray solution. The concentration of hexanal in the final solution used for spraying (0.01%, v/v) was 1 mM. The diluted solution appeared light milky white comprising nanomicelles.

Fruit were sprayed at a rate of 10 L per treatment using a knap-sack sprayer to obtain a good coverage until solution run off was observed. Fruit were subjected to 4 different spray treatments (once a week or twice a week with hexanal formulation, and once a week or twice a week with EFF), carried out for 3 consecutive weeks (a total of 3 sprays over 3 weeks in the case of once a week and a total of 6 sprays (2 sprays/week) over 3 week time) for twice a week treatments. In the case of the twice a week spray treatment, the first spraying was carried out on the first day of the week followed by second spraying on the fourth day. Fruit were harvested a week after spraying (1 week after 1st spray in the twice a week treatment). The experimental trials were arranged in a completely randomized design with 12 plots of ten plants each. There were 4 treatments and three replicates of ten plants per treatment. Only five to six fruit that were set early were allowed to grow on each flower stalk. Fruit from plants not subjected to spraying served as control and were in an adjacent room to protect tomatoes from exposure to hexanal vapour. Three replicates of
control consisting of three plots of 10 plants per plot were maintained. After each treatment, fruit (the entire bunch of 5–6 fruit which is the most mature cluster) were harvested once, after one week of initiation of spray for 3 consecutive weeks (Fig. 3.1). Two clusters of fruit were harvested from each experimental plot (a total of 6 clusters per treatment). After harvesting, all samples were stored at 15°C and after 3 days of storage, samples were brought to room temperature for 12 h prior to evaluating colour and firmness for each harvesting period. Clusters in a treatment were pooled and tomatoes of uniform ripeness selected from clusters were used for further studies. Whole tomatoes were homogenized in a blender for 3 min. The homogenate was then frozen at -20°C and stored until analyzed for other quality parameters.

3.3.2.2 Postharvest dip application of hexanal and EFF

Mature green healthy tomatoes of uniform size, harvested from unsprayed plants were dipped in an aqueous solution containing 0.02% (v/v) hexanal and 0.02% (v/v) EFF for 2.5 min, both containing 2 mM hexanal. Composition of EFF and hexanal stock solutions was as given above (Section 3.3.2.1). The experimental design was completely randomized having three replications of 15 fruit each. Control tomatoes were not dip treated. After dipping, fruit were air dried at room temperature and transferred to a refrigerated incubator maintained at 12°C. Fruit samples were brought out of incubators on 7, 14 and 21 days after treatment and left at room temperature for 12 h prior to measurement of quality parameters of fruit. Whole tomatoes were homogenized in a blender for 3 min. The homogenate was then frozen at -20°C and stored until analyzed for other quality parameters.
Fig. 3.1. A representative picture of tomatoes harvested after one week of initiation of spray for 3 consecutive weeks. Control – 1st batch (A), 1% hexanal once a week – 1st batch (B), 1% hexanal twice a week – 1st batch (C), 1% EFF once a week – 1st batch (D), 1% EFF twice a week – 1st batch (E), Control – 2nd batch (F), 1% hexanal once a week – 2nd batch (G), 1% hexanal twice a week – 2nd batch (H), 1% EFF once a week – 2nd batch (I), 1% EFF twice a week – 2nd batch (J), Control – 3rd batch (K), 1% hexanal once a week – 3rd batch (L), 1% hexanal twice a week – 3rd batch (M), 1% EFF once a week – 3rd batch (N), 1% EFF twice a week – 3rd batch (O). Pictures were taken soon after harvest.
3.3.3 Analysis of quality parameters

3.3.3.1 Colour measurements

Colour of tomato fruits was measured by a Minolta Colour meter (Model CR-300 Minolta, Ramsay, NJ) calibrated with a white standard tile (L = 97.1, $a^+ = 0.29$, and $b^+ = 1.82$). Chromaticity parameters $L$ (brightness), $a^+$ or $a^-$ (red or green), $b^+$ or $b^-$ (yellow or blue) were measured according to the CIE Lab system. Hue angle ($H^\circ$) was calculated as $H^\circ = \tan^{-1}(b^+/a^+)$ degrees and Chroma was calculated as $[(a^-)^2 + (b^-)^2]^{1/2}$ (Igle and Kabelka, 2009). Readings were recorded from both sides of the tomato skin from 5 tomatoes per replicate.

3.3.3.2 Firmness measurements

Firmness of tomatoes was measured by an Ottawa Texture Measuring System (Canners Machinery, Model MC 1061, Simcoe, Canada). Tomatoes were placed on the stage on its pedicel end and firmness was measured by flesh compression to a depth of 1 mm. Results were expressed in Newton/mm (N/mm) and represent the mean of fifteen individual measurements from three replicates of five tomatoes each.

3.3.3.3 Ascorbic acid

The ascorbic acid content of the samples were estimated by titration method using 2,6-dichlorophenol indophenol as an indicator dye (Pelletier, 1985; Rangana, 1986; Nielsen, 1998; Sood and Malhotra, 2001). One percent metaphosphoric acid (HPO$_3$) was prepared by dissolving 30 grams pellets of HPO$_3$ in a volumetric flask with distilled water. Ascorbic acid standard was prepared by dissolving 100 mg of L-ascorbic acid (weigh and dry under sulphuric acid for 24 h in a desiccator) in a volumetric flask with 100 mL of 1% HPO$_3$. One milliliter of this solution was further diluted with 9 mL 1% HPO$_3$ and samples of 10 mL in
three replicates were taken and titrated with 0.025% 2,6-dichlorophenol indophenol to get light pink color, recorded the volume of dye used for standard. To prepare the dye, 50 mg of sodium salt of 2,6-dichlorophenol indophenol was dissolved in approximately 150 mL of warm water containing 42 mg of sodium bicarbonate, cooled and diluted with distilled water to 200 mL, stored in a brown bottle at 3°C. The dye factor is expressed in mg ascorbic acid per mL dye.

Fifty grams of blended tomato slurry was homogenized in 50 mL of 1% HPO₃ and 5 mL of EDTA using small probe of Polytron® Homogenizer (Brinkmann Homogenizer, Switzerland). The homogenate was centrifuged at 8500 rpm for 5 min and filtered the supernatant through glass wool in a funnel. Fifty milliliters of the supernatant was diluted to 100 mL with 1% HPO₃ in a volumetric flask. Pipetted 10 mL aliquots of extract in three replicates and were titrated with standardized 0.025% 2,6-dichlorophenol indophenol to a pink end point lasting for at least 15 s. The calculations were made by using the formula as:

\[
\text{Ascorbic acid (mg/100 g)} = T \times V \times D
\]

Where, \( T \) = ascorbic acid equivalent of dye solution (mg/mL of dye); \( V \) = net ml dye used for titration of aliquot of diluted sample; \( D \) = dilution factor.

### 3.3.3.4 Soluble Solids (°Brix)

Soluble solids contents of juice from blended tomato slurry were measured at room temperature with a hand-held prism refractometer (Fisher Scientific Co., ON, Canada). Results were expressed as (%) soluble solids.
3.3.3.5 pH measurements

The pH of all samples was measured at room temperature with a pH meter (Fisher Scientific Co., ON, Canada).

3.3.3.6 Organic acid

Five grams of blended tomato were weighed accurately into 250 ml beaker and 100 ml double distilled water was added. The resulting mixture was titrated with 0.1 N NaOH to get a pH value of 8.0 – 8.02 with AB15-pH meter (Fisher Scientific Co. ON, Canada). The acidity was calculated as percentage of citric acid on a fresh weight basis (Nielsen, 1998).

3.3.3.7 Effect of hexanal formulations on tomato yield

In pre-harvest spray application the yield was calculated using the amount of total harvests. Ripe tomatoes from each plot were harvested and weighed. The yield (kg m⁻²) from individual plot (2.86 m²) was extrapolated to a per hectare basis. While calculating the yield for post-harvest dip application, tomatoes from all the trials were weighed on 7, 14, and 21 days after treatment.

3.3.3.8 Effect of hexanal formulations on fruit shelf life

To study the effect of postharvest dipping of tomatoes in hexanal (0.02%, v/v) and EFF (0.02%, v/v) on shelf-life, dip treated tomatoes were stored at 12°C for 4 weeks followed by 1 week storage at room temperature prior to analysis. Twelve fruit were used for each treatment. Physical appearance of fruit was examined carefully to determine the effect of treatment on their condition.
3.3.4 Statistical analysis

Experimental means were subjected to an analysis of variance. Means were compared following Tukey’s test using general linear models (GLM) procedure of SAS® software (SAS System, 2002–2008, version 9.2, SAS Institute Inc., Cary, NC, USA). To compare means of values from treated tomatoes with those from control sets, a type I error rate $P < 0.05$ was used for all trials.

3.4 Results

In this study, the effect of hexanal and EFF on quality parameters of tomato fruits were investigated during the pre- and post-harvest treatments. In pre-harvest trials, tomato plants were sprayed with hexanal and EFF once a week and twice a week, with total of three applications of once a week and six applications of twice a week spray schedule. First batch of fruits was harvested one week after the initiation of spray for three consecutive weekly harvestings. In post-harvest applications, mature green tomato fruits were dipped in hexanal and EFF, and stored at $12^\circ$C for 7, 14, and 21 days.

3.4.1 Effect of preharvest treatments on quality parameters of tomatoes

3.4.1.1 Colour parameters of fruit

Tomato fruit color is one of the most important and complex attributes of fruit quality and is the first sensory attribute consumers use to judge the acceptability. The complexity of tomato colour is due to the presence of a diverse carotenoid pigment system, their appearance being conditioned by pigment types and concentrations. Changes in color intensity and quality are important indicators of maturity and quality for fresh tomatoes and development of red color is considered an index of maturity. Four values ($L$, $a^*$, $b^*$ and $H^*$)
were used to identify tomato fruit color. Under conditions where both red intensity and yellow intensity change, the changes in hue angle will be a more reliable parameter to express the increase in red intensity relative to yellow intensity, a decreasing hue angle indicating an increase in red intensity.

Hexanal formulation and EFF were applied as preharvest sprays, once a week, and twice a week on tomato fruit on the vine, for 3 consecutive weeks. Table 3.1 shows colour properties of tomatoes harvested after 2 and 3 weeks of spraying. Brightness was significantly less in tomatoes harvested after 2 weeks of spraying with EFF (twice a week) than other treatments and in the controls (Table 3.1). Brightness was slightly less in the tomatoes harvested after weeks of spraying with hexanal (twice a week) than in the control. The differences in brightness were not significant after 3 weeks of spraying. Fruit harvested 2 weeks after spraying with either hexanal, or EFF, showed increased $\alpha^+$ values suggesting improved red colour than in control fruit (18.03) in all treatments except in the case of tomatoes treated with 0.01% hexanal (once a week, 16.71). An overall reduction in red colour intensity was observed in fruit harvested after 3 weeks of spraying, compared to those subjected to 2 weeks of treatment with hexanal. In contrast to a reduction in red colour intensity, there were no significant differences in yellow colour intensity in response to hexanal and EFF treatments. Hue angle ($H^0$) decreased with increase of red colour intensity ($\alpha^+$) and unsprayed controls recorded higher values than sprayed fruit (Table 3.1). In general, while there was a noticeable reduction in red colour intensity in fruit sprayed with hexanal alone (once a week and twice a week after spraying), EFF sprayed fruit appeared to develop a slightly higher red colour intensity. The chroma values showed a similar trend to $H^0$ and did not show any significant differences between treatments (data not shown).
Table 3.1. Effect of pre-harvest spraying of aqueous formulations of hexanal (1 mM) and EFF (containing 1 mM hexanal) on colour parameters of tomato fruit harvested after two and three weeks of spraying. The data shown are the mean ± standard error from three replicates of five tomatoes each and statistically significant (p < 0.05) values within the columns are designated by different letters. Line across the columns showing individual week analysed separately.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Colour parameters</th>
<th>L(Brightness)</th>
<th>a*(Red)</th>
<th>b*(Yellow)</th>
<th>H*(Hue angle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 2 week (untreated)</td>
<td></td>
<td>41.96 ± 0.63</td>
<td>18.03 ± 0.81</td>
<td>24.39 ± 0.40</td>
<td>55.13 ± 1.50</td>
</tr>
<tr>
<td>Hexanal 2 week (once a week)</td>
<td></td>
<td>41.43 ± 0.36</td>
<td>16.71 ± 0.36</td>
<td>23.99 ± 0.35</td>
<td>55.09 ± 0.66</td>
</tr>
<tr>
<td>Hexanal 2 week (twice a week)</td>
<td></td>
<td>39.04 ± 0.46</td>
<td>21.71 ± 0.35</td>
<td>23.69 ± 0.33</td>
<td>47.51 ± 0.57</td>
</tr>
<tr>
<td>EFF 2 week (once a week)</td>
<td></td>
<td>39.24 ± 0.21</td>
<td>23.34 ± 0.31</td>
<td>23.62 ± 0.26</td>
<td>45.15 ± 0.41</td>
</tr>
<tr>
<td>EFF 2 week (twice a week)</td>
<td></td>
<td>38.59 ± 0.34</td>
<td>22.77 ± 0.51</td>
<td>22.54 ± 0.32</td>
<td>44.29 ± 0.38</td>
</tr>
<tr>
<td>Control 3 week (untreated)</td>
<td></td>
<td>42.40 ± 0.43</td>
<td>13.44 ± 1.73</td>
<td>23.21 ± 0.39</td>
<td>64.20 ± 2.41</td>
</tr>
<tr>
<td>Hexanal 3 week (once a week)</td>
<td></td>
<td>42.55 ± 0.48</td>
<td>13.94 ± 1.11</td>
<td>24.67 ± 0.39</td>
<td>61.99 ± 2.22</td>
</tr>
<tr>
<td>Hexanal 3 week (twice a week)</td>
<td></td>
<td>41.69 ± 0.47</td>
<td>15.95 ± 1.09</td>
<td>24.96 ± 0.37</td>
<td>58.54 ± 2.13</td>
</tr>
<tr>
<td>EFF 3 week (once a week)</td>
<td></td>
<td>41.50 ± 0.46</td>
<td>18.78 ± 1.13</td>
<td>24.73 ± 0.41</td>
<td>54.50 ± 2.04</td>
</tr>
<tr>
<td>EFF 3 week (twice a week)</td>
<td></td>
<td>39.84 ± 0.35</td>
<td>22.10 ± 0.77</td>
<td>24.26 ± 0.31</td>
<td>48.30 ± 1.34</td>
</tr>
</tbody>
</table>

3.4.1.2 Effect of treatments on fruit firmness

Effectiveness of hexanal and EFF spray treatments appeared to increase with time. Firmness of fruit was nearly similar in all treatments when these were harvested after one week (Fig. 3.2). After 2 weeks, no significant differences were noticed in the firmness, though tomatoes subjected to hexanal and EFF sprays twice a week showed slightly rising trend in firmness. In general, fruit subjected to various treatments of hexanal and EFF harvested after 3 weeks of spraying showed the highest firmness values compared to those treated in the first and the second weeks. Highest firmness value of 6.76 N/mm was recorded for tomatoes treated with 1% EFF (twice a week) and harvested after 3 weeks of spraying, indicating a 100% increase in firmness with respect to that of untreated tomatoes followed by 5.50 N/mm and 5.10 N/mm with 1% EFF (once a week) and 1% hexanal (once a week), respectively (Fig. 3.2).
Fig. 3.2. Effect of pre-harvest spraying of aqueous formulations of hexanal (1 mM) and EFF (containing 1 mM hexanal) on tomato fruit firmness (N/mm) harvested after one, two and three weeks of spraying. The data shown are mean ± standard error of three replicates, each containing five fruits. Different letters on the bars indicate statistical significance (p < 0.05), with individual week analysed separately.

3.4.1.3 Effect of preharvest treatments on soluble solids (°Brix)

Soluble solids are very important characteristics for specialty tomatoes quality, and if the taste does not meet consumer’s expectations, they react negatively. Soluble solids contents of tomato fruit sprayed with either hexanal formulation or EFF and harvested after the first and the second weeks were not significantly different (Fig. 3.3). Tomatoes harvested after 3 weeks of spraying with hexanal formulation (once, and twice a week) as well as EEF (once, and twice a week) showed significantly higher (p < 0.05) soluble solids contents compared to those of control fruit. After 3 weeks of spraying, tomatoes treated with hexanal (twice a week) showed 26% higher soluble solids (4.97) followed by 4.80 with EFF
(twice a week) and 4.70 with hexanal, and EFF (once a week) compared to 3.93 °Brix levels of control fruits (Fig. 3.3).

![Graph showing soluble solids content (%) of tomato fruit harvested after different spraying treatments.](image)

**Fig. 3.3.** Effect of pre-harvest spraying of aqueous formulations of hexanal (1 mM) and EFF (containing 1 mM hexanal) on soluble solids content (% soluble solids) of homogenates of tomato fruit harvested after one, two and three weeks of spraying. The data shown are mean ± standard error of three replicate homogenates, each obtained from 100 g of fruit. Different letters on the bars indicate statistical significance (p < 0.05), with individual week analysed separately.

### 3.4.1.4 Effect of preharvest treatments on ascorbic acid levels

Ascorbic acid (Vitamin C) levels were variable in control fruit and those subjected to hexanal and EFF spray treatments (Fig. 3.4). Control fruit showed a declining trend in ascorbic acid levels after the second and third week of harvest. Tomato fruit subjected to spray treatment with hexanal formulation twice a week, showed a gradual and significant increase in ascorbic acid levels from 22 to 47 mg/100 g fresh weight, during the third week of harvest. EFF spray application, once a week, also enhanced the ascorbic acid content of fruit during the second and third weeks of harvest.
**Fig. 3.4.** Effect of pre-harvest spraying of aqueous formulations of hexanal (1 mM) and EFF (containing 1 mM hexanal) on ascorbic acid levels (mg/100 g FW) of tomato fruit harvested after one, two and three weeks of spraying. The data shown are mean ± standard error of three replicate homogenates, each obtained from 100 g of fruit. Different letters on the bars indicate statistical significance (p < 0.05), with individual week analysed separately.

3.4.1.5 Effect of preharvest treatments on organic acids and pH

Organic acid was measured as titratable acidity and expressed as % citric acid. The pH and citric acid in all the samples were determined in a filtrate made from blended tomato fruits. No major differences in citric acid content values were observed after the first and second week of harvest between tomatoes subjected to spraying with the hexanal formulation and the EFF as compared to untreated tomatoes. After 3 weeks of spraying, tomatoes treated with EFF (twice a week) showed significantly lower citric acid as compared to control. In general, citric acid contents were between 0.35 and 0.45% on a fresh weight basis (Table 3.2). The pH was in the range of 4.0–4.5 and not statistically significant (data not shown).
Table 3.2. Effect of pre-harvest spray applications of aqueous formulations of hexanal (1 mM) and EFF (containing 1 mM hexanal) on citric acid levels (%) of homogenates of fruit harvested after one, two and three weeks of spraying. The Data shown are mean ± standard error of three replicate homogenates, each obtained from 100 g of fruit. Statistically significant (p < 0.05) values within the columns are designated by different letters, with individual week analysed separately.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Citric acid levels of tomatoes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of harvest after initial spraying</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>0.41 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hexanal once a week</td>
<td>0.41 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hexanal twice a week</td>
<td>0.42 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EFF once a week</td>
<td>0.45 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EFF twice a week</td>
<td>0.45 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

3.4.1.6 Effect of preharvest treatments on tomatoes yield

The yield was calculated by adding the total weight of harvested tomatoes from each treatment plot for every harvesting batch separately. All the treatments did not appear to have change in yield and fruit weight. In general, average fruit weight was recorded between 96.48 to 98.23 g (Table 3.3), and the yield was observed as 1.20 to 1.23 kg m<sup>-2</sup> (Table 3.4) based on the plot size for each harvesting time.

Table 3.3. Effect of pre-harvest spray applications of aqueous formulations of hexanal (1 mM) and EFF (containing 1 mM hexanal) on average fruit weight (g) of tomatoes harvested after one, two and three weeks of spraying. The Data shown are mean ± standard error of three replicates, each containing ten fruits. Statistically significant (p < 0.05) values within the columns are designated by different letters, with individual week analysed separately.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Av. fruit weight of tomatoes (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of harvest after initial spraying</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>96.93 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hexanal once a week</td>
<td>96.48 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hexanal twice a week</td>
<td>97.01 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EFF once a week</td>
<td>97.25 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EFF twice a week</td>
<td>97.19 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 3.4. Effect of pre-harvest spray applications of aqueous formulations of hexanal (1 mM) and EFF (containing 1 mM hexanal) on average yield (kg m$^{-2}$) of tomatoes harvested after one, two and three weeks of spraying. The Data shown are mean ± standard error of three replicates, each containing ten fruits. Statistically significant (p < 0.05) values within the columns are designated by different letters, with individual week analysed separately.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Av. yield of tomatoes (kg m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of harvest after initial spraying</td>
</tr>
<tr>
<td>Control (untreated)</td>
<td></td>
</tr>
<tr>
<td>Hexanal once a week</td>
<td></td>
</tr>
<tr>
<td>Hexanal twice a week</td>
<td></td>
</tr>
<tr>
<td>EFF once a week</td>
<td></td>
</tr>
<tr>
<td>EFF twice a week</td>
<td></td>
</tr>
</tbody>
</table>

3.4.2 Effect of postharvest treatments on quality attributes of tomatoes

3.4.2.1 Effect of postharvest treatments on fruit colour

The effect of hexanal formulation and EFF on slowing down senescence in tomato fruit was reflected in color parameters $L$ (brightness), $a^+$ (red color intensity), $b^+$ (yellow color intensity) and $H^0$ (hue angle, a relative ratio of the yellow intensity to red intensity expressed as $\tan^{-1}(b^+/a^+)$). Effects of dip treatments on colour parameters of fruit are shown in Table 3.5. There were no significant differences in brightness ($L$) of control fruit and those subjected to dipping in hexanal formulation and EFF during 1–3 weeks of storage. Control fruit started ripening as revealed by an increase in red colour during analysis after 7 days of storage (from $−9.96$ to $0.17$). Red colour intensity in fruit dipped in hexanal formulation or in the EFF ($−6.20$, and $−7.33$ respectively) was similar to that of harvested fruit (0 day). Red colour intensity started to increase with increasing duration of storage, with control fruit showing maximum increase in intensity (from $0.89$ on day 14 to $16.5$ on day 21). In fruit dipped in hexanal formulation and the EFF, increase in red colour was considerably delayed, and reached values in the range of $−5$ to $−6$ on day 14, and $9–9.6$ on day 21 (Table 3.5). By contrast to the red colour values, there were no changes in the yellow colour values ($b^+$) of
the control and treated tomatoes throughout the storage period. A gradual increase in $a^+$ value was also reflected in hue angle ($H^o$) as it decreased during storage. Hue angle values of the hexanal and EFF treated fruit stored for 21 days were 22 and 27% higher than that of control fruit (57.15), suggesting lower levels of red colour development (Table 3.5). Chroma values ranged from 20 to 29 in various treatments and did not show clear trends in response to treatments or storage time (data not shown).

### Table 3.5. Effect of postharvest dip treatment of tomato fruit in aqueous formulations of hexanal (2 mM) and EFF (containing 2 mM hexanal) on colour parameters of tomato fruit, stored at 12°C for 7, 14 and 21 days after treatment and brought out to room temperature for 12 h. Data shown are mean ± standard error of three replicates of five tomatoes each and the statistically significant ($p < 0.05$) values within the columns are designated by different letters. Lines across the columns showing individual time point analysed separately.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Days stored (after dip)</th>
<th>Color parameters</th>
<th>L(Brightness)</th>
<th>$a^+$(Red)</th>
<th>$b^+$(Yellow)</th>
<th>$H^o$(Hue angle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td></td>
<td>57.38 ± 0.33$^b$</td>
<td>-9.96 ± 1.20$^b$</td>
<td>20.97 ± 1.86$^a$</td>
<td>118.14 ± 0.88$^b$</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td></td>
<td>48.50 ± 0.97$^a$</td>
<td>0.17 ± 0.70$^a$</td>
<td>20.15 ± 0.43$^a$</td>
<td>102.10 ± 0.58$^a$</td>
</tr>
<tr>
<td>Hexanal</td>
<td>7</td>
<td></td>
<td>48.68 ± 0.46$^a$</td>
<td>-6.20 ± 1.51$^b$</td>
<td>21.15 ± 0.38$^a$</td>
<td>103.60 ± 3.23$^a$</td>
</tr>
<tr>
<td>EFF</td>
<td>7</td>
<td></td>
<td>49.05 ± 0.30$^a$</td>
<td>-7.33 ± 0.61$^b$</td>
<td>22.24 ± 0.44$^a$</td>
<td>106.40 ± 2.28$^a$</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td></td>
<td>47.41 ± 0.61$^a$</td>
<td>0.89 ± 0.15$^a$</td>
<td>21.86 ± 0.78$^a$</td>
<td>94.02 ± 4.50$^a$</td>
</tr>
<tr>
<td>Hexanal</td>
<td>14</td>
<td></td>
<td>48.19 ± 0.41$^a$</td>
<td>-5.41 ± 1.23$^b$</td>
<td>22.12 ± 0.41$^a$</td>
<td>95.60 ± 3.03$^a$</td>
</tr>
<tr>
<td>EFF</td>
<td>14</td>
<td></td>
<td>48.27 ± 0.50$^a$</td>
<td>-6.64 ± 0.90$^b$</td>
<td>22.68 ± 0.43$^a$</td>
<td>99.86 ± 2.92$^a$</td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td></td>
<td>43.72 ± 0.55$^a$</td>
<td>16.50 ± 1.14$^a$</td>
<td>24.81 ± 0.28$^a$</td>
<td>57.15 ± 2.01$^a$</td>
</tr>
<tr>
<td>Hexanal</td>
<td>21</td>
<td></td>
<td>44.60 ± 0.88$^a$</td>
<td>9.63 ± 1.74$^b$</td>
<td>25.32 ± 0.58$^a$</td>
<td>69.91 ± 3.61$^a$</td>
</tr>
<tr>
<td>EFF</td>
<td>21</td>
<td></td>
<td>46.74 ± 0.59$^a$</td>
<td>9.03 ± 1.65$^b$</td>
<td>24.59 ± 0.34$^a$</td>
<td>72.35 ± 3.44$^a$</td>
</tr>
</tbody>
</table>

#### 3.4.2.2 Effect of postharvest treatments on fruit firmness

Firmness of tomato fruits was decreased gradually with the passage in storage (Table 3.6). As noticed in the spray treatment, fruit firmness was affected by dip treatment of fruit with hexanal formulation and EFF. Firmness of mature green fruit was 13.90 N/mm on day 0, which decreased to 6.3 N/mm on day 7, to 4.3 N/mm on day 14, and to 3.5 N/mm on day 21 of storage, showing a significant decline in firmness during storage (Table 3.6).
Postharvest treatments of tomatoes with hexanal formulation and EFF significantly reduced the decline in firmness observed after 14 and 21 days of storage. Only a slight variation in fruit firmness of tomatoes subjected to postharvest treatments was recorded in the initial 7 days of storage. At later stages of storage (14 and 21), this firmness difference between control and treatments were clearly evident. As shown in Table 3.6, fruit dipped in 2 mM EFF were significantly firmer (5.3 and 5.51 N/mm respectively) than the control (4.3 and 3.52 N/mm respectively) after 14 and 21 days of storage.

### 3.4.2.3 Effect of postharvest treatments on ascorbic acid levels

Initial levels of ascorbic acid on day 0 was 21.90 mg/100 g fresh weight, and as shown in Table 3.6, ascorbic acid content of fruit decreased during storage, and the decrease was considerably higher in untreated fruit. Initially, on day 7 of storage, no significant differences in ascorbic acid levels were observed in all the treatments. After 14 and 21 days in storage, fruit subjected to hexanal dip treatments had ascorbic acid levels at 9.03 and 7.17 mg/100 g respectively, while those subjected to EFF dip treatment showed higher levels of ascorbic acid at 12.63 and 16.13 mg/100 g respectively, than untreated fruit (7.10) and 6.70 mg/100 g respectively) (Table 3.6). Ascorbic acid content in fruit dipped in 0.02% EFF (16.13 mg/100 g) after 21 days in storage was nearly 141% higher (p < 0.05) than that of undipped controls (6.70 mg/100 g).

### 3.4.2.4 Effect of postharvest treatments on soluble solids and organic acids

There were no significant differences in soluble solids and organic acid contents between undipped control tomatoes and those that were dipped in hexanal formulation and EFF during storage (Table 3.6). Soluble solids contents remained in the range of 4–4.5% in
control as well as in dip treated tomatoes. Acidity levels in treated and untreated tomatoes decreased gradually during storage. Organic acid contents varied between 0.42 and 0.57% on fresh weight basis, during the entire period of storage (Table 3.6). The pH was in the range of 4.25–4.50 throughout storage time (data not shown).

Table 3.6. Effect of postharvest dip treatment of tomato fruit in aqueous formulations of hexanal (2 mM) and EFF (containing 2 mM hexanal) on quality parameters of tomato fruit, stored at 12°C for 7, 14 and 21 days after treatment and brought out to room temperature 12 h prior to homogenization. Firmness data shown are from three replicates of five tomatoes each. Other data shown are mean ± standard error of three replicate homogenates obtained from 100 g of tomatoes each. Statistically significant (p < 0.05) values within the columns are designated by different letters. Lines across the columns showing individual time point analysed separately.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Days stored (after dip)</th>
<th>Quality parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Firmness (N/mm)</td>
<td>Soluble solids (%)</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>13.90 ± 1.31 b</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>6.36 ± 0.44 a</td>
</tr>
<tr>
<td>Hexanal</td>
<td>7</td>
<td>6.40 ± 0.37 a</td>
</tr>
<tr>
<td>EFF</td>
<td>7</td>
<td>6.12 ± 0.26 a</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>4.30 ± 0.38 b</td>
</tr>
<tr>
<td>Hexanal</td>
<td>14</td>
<td>5.20 ± 0.16 a</td>
</tr>
<tr>
<td>EFF</td>
<td>14</td>
<td>5.30 ± 0.18 a</td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>3.52 ± 0.21 c</td>
</tr>
<tr>
<td>Hexanal</td>
<td>21</td>
<td>4.60 ± 0.26 b</td>
</tr>
<tr>
<td>EFF</td>
<td>21</td>
<td>5.51 ± 0.48 a</td>
</tr>
</tbody>
</table>

3.4.2.5 Effect of postharvest treatments on shelf-life of tomatoes

Tomatoes were visually examined for the presence of any infection, spoilage and wilting to assess the effects of hexanal- and EFF-dipping on shelf-life after 4 weeks of storage at 12°C followed by 1 week storage at room temperature. Quality of hexanal and EFF dipped tomatoes were superior to undipped ones with respect to colour, firmness and visual appearance. Control tomatoes were over-ripened, soft and spoilage had started to
set in. Between the hexanal and EFF treatments, EFF dipped tomatoes were the ones that stayed fresher and brighter without any visible bruises and spoilage. While fruit dipped in hexanal formulation showed some spoilage, the condition of fruit was better than the control (Fig. 3.5).

![Image of tomato fruit shelf-life experiment](image)

**Fig. 3.5.** Effect of postharvest dip treatment of hexanal and enhanced freshness formulation (EFF) on tomato fruit shelf-life. Control (left panel), treated with 0.02% (2 mM) hexanal (middle panel); and treated with 0.02% (2 mM) hexanal as EFF (right panel). Tomatoes were stored at 12°C for 4 weeks followed by further storage at room temperature (25°C) for 1 week. All samples were photographed after 5 weeks of initiating the experiment. Four of the control tomatoes were damaged after 5 weeks, only 8 of the original 12 fruit are shown.

### 3.5 Discussion

The post-harvest life of fruit is now often characterized by the notion of senescence as an overriding biological phenomenon, of which ripening is an integrated part (Brady, 1987). In
fact, some ripening syndromes can hasten the senescence process, with pigment evolution and early deterioration of chloroplast envelopes and thylakoids (Mazliak, 1987). Fruit ripening is a dynamic, transitional, metabolically active phase which involves several biochemical processes such as break down of cell walls and pectin, degradation of membranes, breakdown of stored carbohydrates into sugars, a reduction in acidity and an increase in biosynthesis of colour and volatile aroma components, all contributing to an overall improvement in organoleptic quality of fruit (Paliyath et al., 2012). The shelf life of many fresh-cut leafy and root products has been extended successfully, but for fruit that continue to ripen after harvest the results are far from satisfactory (Beaulieu and Gorny, 2001). A similar situation is encountered with fruit such as tomatoes. Although tomato is a natural ingredient in many salads, its use in ready-to-eat salads is still very restricted due to its short shelf-life when minimally processed. The use of fresh-cut tomato by fast-food restaurants, food service institutions, and cafeterias is also limited by the many technical problems in maintaining its quality and microbiological safety during storage (Hong and Gross, 2002). Application of hexanal compositions is a relatively new technology shown to be effective in extending shelf-life and quality of several fruit such as apple, cherry, peach, plum, vegetables and flowers (Paliyath and Murr, 2007; Sharma et al., 2010; Tiwari and Paliyath, 2011a). Hexanal is produced naturally during lipid peroxidation mediated by lipoxygenase and hydroperoxide lyase, and has the characteristic green flavour produced during wounding processes in green plants. It has GRAS (generally regarded as safe) status (Paliyath and Subramanian, 2008). Formulations containing hexanal can be applied as a dip, spray, and as a vapour (Paliyath and Subramanian, 2008). Hexanal does not accumulate in tissue, as it gets converted to hexanol and can get further metabolized through the respiratory cycle.
The present study was an attempt to determine effects of pre-and postharvest applications of hexanal and EFF on quality parameters of greenhouse tomatoes. Since effectiveness of hexanal application is dependent on physiological maturity, optimal effects have been observed when fruit are treated well in advance of ripening, and this effect may differ from fruit to fruit. In tomato, optimum effectiveness was observed when fruit were dipped in hexanal formulation at the mature green stage, which also coincides with the temporal increase in PLD activity that occurs during advancement in ripening (Pinhero et al., 2003). As greenhouse tomatoes are non-determinate, preharvest spray applications will expose fruit at various stages of development to hexanal and other ingredients. Hexanal was applied as a spray or as a dip to mature green tomatoes, and its overall effects were assessed by monitoring changes in shelf-life and quality parameters. In this study, we have observed that hexanal formulations showed better results with multiple spray applications rather than single foliar treatment. Hexanal single dose treatment has no significant effects on physiology and quality attributes in tomato fruits (Utto et al., 2008).

Previous work has conclusively demonstrated the beneficial effects of hexanal formulation dips on fresh eating tomatoes (Tiwari and Paliyath, 2011a). A comparison of changes in gene expression induced by hexanal and 1-MCP showed that hexanal selectively down-regulated expression of several genes that are activated during ripening and senescence, by contrast to the almost global down-regulation of genes induced by 1-MCP treatment. Hexanal treatment resulted in down-regulation of genes involved in ethylene biosynthesis and signal transduction processes, cell wall breakdown, lipid metabolism etc., without affecting those involved in the development of quality characteristics (Tiwari and Paliyath, 2011a). Changes in colour intensity and quality are important indicators of maturity and quality for fresh tomatoes and development of red colour is considered as an index of maturity (Lopez Camelo and Gomez, 2004). In
preharvest treatments, red colour intensity was slightly higher in tomatoes sprayed with EFF than those sprayed with hexanal formulation, suggesting that hexanal formulation may be more effective in delaying ripening. It is interesting to note that the yellow colour component that predominantly indicates the level of carotenoids (Itle and Kabelka, 2009), remained nearly constant, irrespective of treatments, suggesting that the carotenoid biosynthetic pathway was unaffected by the treatments. Previous studies have demonstrated that lycopene is converted to β-carotene by the enzyme sesquiterpene cyclase during development of fruit. Once ripening is activated, sesquiterpene cyclase levels are reduced which leads to the accumulation of lycopene, and red colour (Ronen et al., 1999; Srivastava and Handa, 2005). Therefore, reduced red colour intensity in hexanal formulation sprayed tomatoes is a clear indication that ripening processes are inhibited. In an earlier study, Sharma et al. (2010) noticed an increase in bright red colour in cherries harvested from 0.02% EFF sprayed trees in contrast to unsprayed and hexanal formulation sprayed trees, suggesting a developmental delay. Effectiveness of hexanal and EFF on slowing down senescence was also reflected in postharvest dip applications. Tomatoes dipped in EFF and hexanal showed higher L values, hue angle, and reduced red colour intensity than control fruit during storage, suggesting a delay in ripening. These results are in agreement with our earlier observations (Tiwari and Paliyath, 2011a) where a postharvest treatment with EFF formulation enhanced shelf-life of fresh eating tomato fruit for over 21 days of storage without any damage.

Next to visual appearance, firmness is one of the most important quality parameters in tomato which is closely associated with ripeness and shelf-life. Most consumers prefer firm fruits which do not lose too much juice when sliced (Kader et al., 1977). It is evident from the data that firmness of tomato fruit was influenced significantly by the application of hexanal and EFF formulation in both pre- and postharvest treatments. Preharvest
applications of 0.01% EFF (twice per week) on tomatoes resulted in higher fruit firmness than those subjected to hexanal treatment or that of control fruit. Tomatoes treated with 0.02% EFF showed the highest firmness, which was retained during 21 days in storage. Hexanal treatment of tomatoes resulted in down-regulation of polygalacturonase and β-galactosidase genes, the expression of which is critical to pectin degradation and fruit softening (Tiwari and Paliyath, 2011a). Similar changes in gene expression may have resulted in the inhibition of pectin degradation resulting in increased firmness, also observed in this study.

Tomato flavour involves perception of taste and aroma of many chemical constituents. Sugars, acids, and their interactions are important to sweetness, sourness, and overall flavour intensity in tomatoes (Stevens et al., 1977). The ratio of soluble solids to organic acids is important to sweetness and sourness of tomatoes. High acidity and low soluble solids contents will produce a tart tomato, while high soluble solids and low acidity will result in a bland taste. When both soluble solids and organic acids are low, the result is a tasteless, insipid tomato. In general, preharvest treatments with hexanal and EFF showed significantly higher soluble solid contents in tomato fruit subjected to spray treatments at the third week of harvest. In this study, pre- and postharvest treatments with hexanal and EFF did not result in major differences in pH and acidity. The pH values and citric acid content were within standard limits and are in line with previous reports (Wang and Vestrheim, 2002; Oke et al., 2003). Additional beneficial effects such as increased ascorbic acid and pathogen resistance have also been observed as a result of hexanal treatments (Utto et al., 2008).
3.6 Conclusion

To conclude, postharvest applications of hexanal and hexanal containing formulations (EFF) hold promise in prolonging postharvest shelf-life and preserving nutritional attributes of tomatoes. As observed in cherry fruit (Sharma et al., 2010), effectiveness of EFF may also be modified by the presence of antioxidants which can provide protection from free radicals if internalized, and delay senescence. The differential effects of hexanal formulation and EFF on the quality characteristics of vine-ripened tomatoes and mature green tomatoes subjected to postharvest dips may arise because of the nutrient channelling and utilization. While the fruit subjected to preharvest spray have a continued nutritional source, those dipped in formulations have to rely on internal stored sources for the biosynthesis of quality components. Shelf-life of tomatoes is considerably low as current methods do not provide an efficient means of maintaining quality. The technology described here provides a safe and efficient strategy for enhancing shelf-life and nutritional quality of tomatoes. It is also noteworthy that no negative effects were observed in developing fruit because of multiple spray treatments with hexanal formulations, potentially suggesting that its effects on gene expression may become more pronounced only when fruit reach physiological maturity.
CHAPTER 4
Volatile generation and release kinetics of hexanal during postharvest hexanal vapour application for enhancing the shelf life and quality of bell peppers

4.1 Abstract

Release kinetics of hexanal during vapour application were measured in the headspace with and without bell pepper at defined intervals using an Agilent Gas Chromatograph (GC) equipped with an MSD detector. Headspace hexanal concentration dropped to 5% of its peak within 3 h of application in the presence of pepper fruit, which indicated a major portion of the applied hexanal is absorbed and metabolized within the tissue. Pepper subjected to 0.01% (w/w) hexanal vapour for 0 – 16 h treatments showed a significant (p<0.05) delay in ripening and enhancement of postharvest qualities when exposed for 6 h and above, even at 28 d of storage. Treated fruits were characterized by decreased red colour development and increased firmness, which indicated a delay in ripening and inhibition of membrane deterioration. Hexanal treatments also resulted in an increase in the volatile compounds in bell peppers compared to untreated samples. Evidence from our study indicated that postharvest hexanal vapour treatment at 0.01% for 6 h effectively enhanced the quality and shelf life of bell peppers and that most of the hexanal was absorbed and metabolized within tissues.

4.2 Introduction

As aroma is one of the most important characteristics for fruit quality, volatile compounds are likely to have a huge effect on the perception and acceptability of fruit products by consumers (El Hadi et al., 2013). Most fruits produce a significant number of volatile compounds and their qualitative and quantitative composition determines fruit aromatic characteristics. Many of these volatile compounds are produced in trace amounts,
which are below the thresholds of most analytical instrument, but can be detected by human olfaction (Zhu et al., 2005; Goff and Klee, 2006; Song and Fornay, 2008; Defilippi et al., 2009). In fruits, some important aroma compounds are derived from fatty acids, amino acids, phenols and terpenoids (Schwab et al., 2008). Among these compounds, volatiles generated from fatty acids via the lipoxygenase (LOX) pathway are responsible for the “green” fresh note, which is desirable in many fruit products (Ho and Chen, 1994). Fruit volatile composition includes an array of chemicals from various classes, such as alcohols, aldehydes, esters, ketones and terpenes, and it plays a major role in the market success of any fruit. The LOX pathway is an enzymatic pathway for lipid oxidation. The main products of the LOX pathway are C₆ aldehydes and alcohols generated through sequential enzymatic reactions. The recognized flavour of a particular type of fruit is usually absent in the early stage of its development and, instead, is acquired during the ripening process as a consequence of volatile accumulation. Volatiles responsible for fruit aroma can be classified as “primary” or “secondary”, indicating whether they are present in intact fruit tissue or produced as a result of tissue disruption. Volatiles collected from intact fruit reflect the consumer smelling and perceiving ripening signals of the fruit, while volatiles generated after tissue disruption may better represent the flavour perception during eating.

In bell peppers the major lipoxygenase (LOX) generated volatiles are \((Z)-3\)-hexenal, \((E)-2\)-hexenal, hexanal, \((Z)-3\)-hexenol, \((E)-2\)-hexenol, and hexanol which are created from two fatty acids, linoleic and linolenic acid. Upon rupturing of the cells the LOX enzyme combines with oxygen and forms hydroperoxides. Hydroperoxides are then converted by hydroperoxide lyase and in linoleic acid this creates hexanal while in linolenic acid it creates \((Z)-3\)-hexenal. Hexanal is then converted into hexanol by alcohol dehydrogenase while \((Z)-3\)-hexenal is converted into two products one being \((Z)-3\)-hexenol by alcohol dehydrogenase and the other product is \((E)-2\)-hexenal by \(Z3/E2\) isomerase. \((E)-2\)-Hexenal
is then converted into (E)-2-hexenol by alcohol dehydrogenase (Luning et al., 1995). In tomato fruits over 400 volatile compounds have been detected, the most important contributor to tomato fruit aroma being hexanal, hexanol, 3-methylbutanal, 3-methylbutanol, methyl nitrobutane and isobutyl thiazole and a general increase in these and other volatiles occur during ripening (Zhu et al., 2005; Birtic et al., 2009). An overwhelming number of chemical compounds have been identified as volatile compounds in fresh fruit, based on their quantitative abundance and olfactory thresholds, although only a fraction of these compounds have been identified as fruit flavour impact compounds. In Table 4.1 some examples of aroma substances and a description of their odour are shown (Díaz-Mula, 2011).

<table>
<thead>
<tr>
<th>Aroma substances</th>
<th>Odour description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>Pungent, penetrating</td>
</tr>
<tr>
<td>Acetone</td>
<td>Sweet, pungent</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Ether-like, pineapple, anise</td>
</tr>
<tr>
<td>Methyl butyrate</td>
<td>Apple</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>Onion, Cabbage</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>Fruity, pineapple</td>
</tr>
<tr>
<td>Butyl acetate</td>
<td>Fruity</td>
</tr>
<tr>
<td>1-Methyl-ethyl butyrate</td>
<td>Apple</td>
</tr>
<tr>
<td>Hexanal</td>
<td>Cut grass</td>
</tr>
<tr>
<td>Hexenal</td>
<td>Sweet, almond, green</td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>Green leaf</td>
</tr>
<tr>
<td>Heptanone</td>
<td>Banana</td>
</tr>
<tr>
<td>Methyl hexanoate</td>
<td>Ether-like, pineapple</td>
</tr>
<tr>
<td>Butyl butyrate</td>
<td>Fruity, pear</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>Fruity</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>Fruity, apricot</td>
</tr>
<tr>
<td>Linalool</td>
<td>Fruity, floral, citrus</td>
</tr>
<tr>
<td>B-lonone-trans</td>
<td>Warm, woody, balsamic, rose</td>
</tr>
</tbody>
</table>

It has been reported by Han (2015) that hexanal concentration in the headspace of bell pepper and tomato fruits after tissue disruption was increased rapidly, and reached maximum concentrations around 5-20 min (Fig. 4.1). In both the cases, the highest LOX
activity and hexanal concentration was at 25°C and the lowest was at 4°C, followed by 45°C. Similar results were found in other studies, where it was reported that LOX activity increased from 0 to 20°C, reached its maximum activity at 20 to 30°C and decreased at higher temperatures in tomato fruits (Jadhav et al., 1972; Yilmaz, 2001).

Hexanal treatment showed promising results in enhancing the shelf life of several fruits, vegetables and flowers such as mango, apples, cherry, peach, strawberry, carnation, roses (Paliyath and Subramanian, 2008; Anusuya et al., 2016), and various vegetables such as tomato, peppers (Cheema et al., 2014; 2018). Previous studies demonstrated the optimal levels of postharvest vapour application that can effectively enhance the quality and shelf life of sweet bell peppers (Cheema et al., 2018). However, the most effective time for hexanal vapour treatments to enhance the shelf life and quality parameters of greenhouse grown peppers is unknown. No studies on the effects of best optimal inhibitory exposure to hexanal vapour with regards to shelf life and quality parameters of fresh produce have been reported in the literature. In this study, the objective was to explore the potential of using hexanal to enhance shelf life and quality parameters of bell peppers using the minimum exposure time to hexanal vapour treatment. In addition, we intended to determine the release kinetics and absorption of hexanal by pepper fruits during vapour application. Finally, we sought to measure the extent to which volatile compound production of pepper fruits was enhanced by the hexanal formulation.

![Fig. 4.1. Effect of temperature on the concentration of hexanal in the headspace of (A) bell pepper and (B) tomato fruits (adapted from Han, 2015).](image)
4.3 Materials and methods

4.3.1 Plant materials

Sweet bell peppers (*Capsicum annum* L. cv. Redline) plants were grown hydroponically (7.2 plants m$^{-2}$ on coconut coir growing media) under natural light conditions in a commercial greenhouse (St. Davids Hydroponics, Niagara, Ontario) following standard production practices (OMAFRA, 2010). Fruits of uniform size, colour and free of disease at the mature green stage were harvested to use for various experiments. Peppers harvested at 0800 h were transported using cardboard boxes and arrived at the laboratory of University of Guelph the same day at 1000 h. This study consisted of two experiments; 1) release kinetics of hexanal and 2) postharvest hexanal vapour treatment of bell peppers. In the second experiment investigations were carried out to determine the best effective time of hexanal vapour treatment and effect of hexanal on volatile compound generation of bell peppers.

4.3.2 Release kinetics of hexanal

A known volume of hexanal solution was pipetted onto a Whatman 3 paper kept inside a small beaker, which was then placed immediately into a clean glass jar. The glass jar was immediately sealed tight with a lid. Head space gas samples were withdrawn using a needle through a septum fitted onto the lid of the container at defined intervals of 20 min for 4 h. The headspace gas samples were analyzed in an Agilent Gas Chromatograph (GC) equipped with a single quadrupole MSD detector, using HP-5 MS column (30 m x 0.25 mm). The profile and pattern of hexanal release was also determined in the presence of pepper fruit as outlined above. The procedure was repeated three times. The amount of hexanal in the headspace gas samples at a given time was calculated using calibration curves for hexanal concentrations in the rage of (1/100, 1/1000 and 1/10,000). Known concentrations
of pure hexanal were analyzed using a GC and the peak areas obtained from the analysis were plotted against the hexanal concentrations.

4.3.3 Postharvest hexanal vapour treatment of bell peppers

In this experiment pepper fruit (Capsicum annuum L. cv. Redline) at the mature green stage were exposed to 0.01% hexanal vapor (based on fruit fresh weight) for various durations of time (0 – 16 h) to determine the best effective time of treatment. Hexanal vapor treatment was conducted according to standard procedures (Cheema et al., 2018). Different batches of pepper fruits were exposed to 0.01% hexanal vapor for 0 - 16 h and control fruit were left untreated (0 h). Peppers were sorted into 6 groups, weighed and each group of fruit were placed in 6 mil plastic bags. Bags were closed and sealed immediately after placing the required amount of hexanal (0.01 % based on fruit fresh weight) measured into a beaker along with a strip of Whatman 3 filter paper to facilitate rapid evaporation. After the hexanal exposure, fruit were removed from bags and stored separately at 12°C, 90–95% relative humidity in refrigerated incubators for 28 days. Each treatment was performed in three replications of 3 fruit per replication. Samples were withdrawn on 1, 7, 14, 21 and 28 days of storage for analysis of various quality parameters such as fruit colour (Minolta Chromameter) and firmness (N) following standard methods (Cheema et al., 2018). Ripening progression and color development in fruit was visually assessed on days 1, 14, 21 and 28.

4.3.4 Analysis of volatile compounds of pepper fruit exposed to hexanal vapour

Untreated and hexanal vapor treated (0.01%) pepper fruit (21 day storage) were separately placed in a glass container and then sealed tightly with a lid connected with a septum for sample collection. Fruit were left in the container for 1 h for head space gas
collection. The volatile compounds in the headspace were analyzed using solid phase microextraction (SPME) gas chromatograph (Agilent Technologies, USA). Volatiles were extracted from the headspace by inserting a 65µm SPME fiber (coated with polydimethylsiloxane) into the headspace through the septum and the fiber was exposed to volatiles for 20 min. The fibre was inserted into a GC (Agilent) injection port and volatiles were desorbed for 7 min at 250°C (inlet temperature) and the volatiles separated using a temperature gradient starting from 50°C to 200°C (rate 10°C/min). Helium at a pressure of 15 psi was used as a carrier gas and the MS source temperature was maintained at 250°C. The spectral data were analysed using Agilent Chemstation. The volatile compounds were identified by comparing mass spectral data of samples with those of the NIST (National Institute of Standards and Technology) mass spectral library.

4.3.5 Statistical analysis

Means were subjected to an analysis of variance. Means separated while following the Tukey’s test using the general linear models (GLM) procedure of SAS® software (The SAS System, 2002–2010, version 9.4, SAS Institute Inc., Cary, NC, USA). To compare means of treated fruit with control fruit, a type I error rate P< 0.05 was used for all trials.

4.4 Results and discussion

4.4.1 Release kinetics of hexanal

The release pattern of hexanal without pepper fruits is depicted in Figure 4.2. It has been observed that after the introduction of hexanal, the maximum concentrations (1.35 ng mL⁻¹) peaked around 30 min and remained constant over the 160 min. The behaviour of hexanal was similar in each replication, increasing or remaining constant over the entire time. Release kinetics of hexanal in the headspace with the presence of pepper fruit was
evaluated and data showed in Figure 4.3. It was observed that on application, hexanal (liquid) evaporated and hexanal concentration reached its maximum levels (2.0 ng mL$^{-1}$) within 30 min after initiation of the treatment. In the first several minutes after sealing the glass jar, hexanal was generated and accumulated about 48% higher in the headspace compared to the hexanal concentration without peppers.

![Graph showing hexanal release pattern without peppers](image)

**Fig. 4.2.** Pattern of hexanal release without pepper fruits during 3.5 h. Headspace gas samples withdrawn at defined intervals were analyzed using gas chromatography. Given data from three replicates indicated as R1, R2 and R3.

This increase in hexanal concentration in the presence of bell peppers (210 g) indicates the property of naturally occurring compound and the production of hexanal by pepper fruits (Hildebrand et al., 1998; Paliyath et al., 2003). Similar hexanal generation peaks in the headspace of bell peppers and tomatoes were noted by Han, (2015); and Xu and Barringer, (2009). We have observed that hexanal concentration in the head space came down to 0.1 ng mL$^{-1}$ after 3 h of incubation which is correspond to 5% of the peak value (2.0 ng mL$^{-1}$). The levels of hexanal were extremely low sensory threshold of
detection concentration of 0.1 ng mL\(^{-1}\) at 180 min (Fig. 4.3). Therefore, most of the hexanal is absorbed and metabolized within the tissue. Thus, a short exposure to hexanal appears to be adequate for enhancing shelf life and quality of bell peppers.

![Graph showing hexanal release over time](image)

**Fig. 4.3.** Pattern of hexanal release in the presence of pepper fruit during 3.5 h. Headspace gas samples withdrawn at defined intervals were analyzed using gas chromatography. Given data from three replicates indicated as R1, R2 and R3.

### 4.4.2 Effect of hexanal treatment time on quality parameters of pepper fruit

#### 4.4.2.1 Effect of treatments on fruit colour

The effect of hexanal treatment time on colour development of postharvest bell pepper is depicted in Table 4.2. The brightness, red intensity, and the yellow intensity were measured using a Minolta colorimeter as \(L\) (lightness), \(a\) (\(a^+\) - red colour intensity; \(a^-\) - green colour intensity) and \(b\) (\(b^+\) - yellow colour intensity). These preliminary data indicated a normal pattern of colour development in untreated peppers (0 h), and a pattern of delayed
ripening in hexanal vapour exposed peppers (e.g. slightly reduced red colour development on day 14). The colour qualities of hexanal treated peppers for 2 h were quite similar to those of control fruits (0 h) during further storage and $a^+$ values gradually increased during the storage which reflected fruit ripening. The untreated fruits (0 h) and fruits exposed to hexanal vapour for 2 h started to develop red colour on day 21 which turned redder by day 28. This is indicated in their $a^+$ values which increased from -8.96 and -8.70 on day 1 to 5.00

Table 4.2. Effect of hexanal vapour treatments on firmness (Newton) and colour parameters of pepper fruits stored for 1, 14, 21 and 28 days in refrigerator incubator at 12°C and 90-95% relative humidity. The data shown are the mean ± standard error from three replicates of three fruits each with three measurements per fruit. Statistically significant (p<0.05) values within the columns are designated by different letters. Lines across the columns showing individual time point analysed separately.

<table>
<thead>
<tr>
<th>Hexanal Treatment (h)</th>
<th>Days after treatment</th>
<th>Firmness (Newton)</th>
<th>Color parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$L$ (Brightness)</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>105.27±0.48a</td>
<td>32.83±0.30a</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>109.13±2.11a</td>
<td>33.05±0.49a</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>108.37±0.52a</td>
<td>33.15±0.26a</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>111.30±2.47a</td>
<td>33.49±0.44a</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>111.33±1.05a</td>
<td>33.14±0.41a</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>110.03±0.62a</td>
<td>33.24±0.12a</td>
</tr>
<tr>
<td>0</td>
<td>14</td>
<td>82.87±1.46b</td>
<td>34.80±0.80dc</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>80.47±1.07b</td>
<td>35.43±0.29dc</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>94.30±0.55a</td>
<td>33.74±0.38d</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>96.47±0.55a</td>
<td>36.48±0.78bc</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>98.07±1.69a</td>
<td>37.91±0.24ab</td>
</tr>
<tr>
<td>16</td>
<td>14</td>
<td>95.80±4.20a</td>
<td>38.63±0.51a</td>
</tr>
<tr>
<td>0</td>
<td>21</td>
<td>72.23±0.90d</td>
<td>35.87±0.21c</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>75.20±0.93c</td>
<td>35.91±0.36c</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>91.17±0.75b</td>
<td>35.89±0.25c</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>93.37±0.49ab</td>
<td>36.86±0.08b</td>
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<tr>
<td>10</td>
<td>21</td>
<td>93.80±0.31a</td>
<td>37.79±0.16a</td>
</tr>
<tr>
<td>16</td>
<td>21</td>
<td>93.73±0.22a</td>
<td>37.91±0.07a</td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td>60.83±0.26c</td>
<td>38.86±0.44a</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>60.80±0.40c</td>
<td>39.18±0.58a</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>75.77±0.64b</td>
<td>34.89±0.25b</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>79.57±0.69a</td>
<td>35.50±0.52b</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>79.50±0.66a</td>
<td>36.02±0.34b</td>
</tr>
<tr>
<td>16</td>
<td>28</td>
<td>80.33±0.54a</td>
<td>35.30±0.21b</td>
</tr>
</tbody>
</table>
and 5.28 respectively, by day 28. Hexanal treated peppers for 4 h just started to develop red color on day 28. By contrast, peppers exposed to hexanal vapour for 6, 10 and 16 h did not develop any red colour during the entire period of storage. Peppers treated with hexanal vapour for 10 and 16 h showed an increase in their brightness compared to the control and other vapour treatments until 21 day of storage, but a decrease in their brightness was noticed by the end of storage period. In our results the increase of $L$ values in control fruits (0 h) and fruits treated with hexanal for 2 h by the end of storage period probably indicated that during fruit ripening fruits become lighter by changing in colour from green to yellow-red as indicated by Kader and Cantwell (2010) in the colour scale for green peppers. Contrary to these results, Pérez- López et al., (2007) found that as luminosity decreases, pepper fruits become darker. Peppers exposed to hexanal for 6 h, 10 h and 16 h showed higher $L$ values and reduced red colour intensity than control fruit (0 h) and fruit exposed to hexanal for 2 h until day 21 of storage, suggesting a delay in ripening. But on the other hand, untreated (0 h) and hexanal vapour treated bell peppers for 2 h showed a significant increase in yellow colour intensity during storage. Reduced red colour intensity and enhanced firmness were clear evidence of delayed ripening in peppers treated with hexanal vapour for 6 h, 10 h and 16 h compared to the control (0 h) and 2 h treatments during their storage. Ripening inhibition, as indicated by the delay in red colour development, increased when the exposure time to hexanal was increased. These observations are similar to our earlier studies where increased concentration of preharvest hexanal spray, and postharvest dip treatments of tomato (Cheema et al., 2014) and postharvest hexanal vapour treatments of bell pepper (Cheema et al., 2018), delayed red colour development and enhanced firmness during storage.
4.4.2.2 Effect of treatments on fruit firmness

A continuous and gradual decrease in fruit firmness was observed in the control (0 h) and peppers treated with hexanal vapour for 2 h during prolonged storage for 28 days. On the contrary, fruit exposed to hexanal vapour for 6 h, 10 h and 16 h showed significantly enhanced firmness throughout the entire storage time (Fig. 4.4). However, no significant differences in firmness were noticed among the vapour treatments for 6 h, 10 h and 16 h and the fruits showed the firmness of 79.57 N, 79.50 N and 80.33 N, respectively by day 28. Fruit treated with hexanal for 4 h showed higher firmness compared to control (0 h) and 2 h exposure time but significantly lower than 6, 10 and 16 h exposure time. Untreated fruit (0 h) and the fruit treated with hexanal vapour for 2 h had a 44% reduction in firmness by the end of the storage period as compared to fruit treated with hexanal vapour for 6 h, 10 h and 16 h, which showed a minor loss in firmness (Table 4.2). These results are in the agreement with earlier studies (Cheema et al., 2014) where preharvest hexanal spray applications and postharvest dip treatments enhanced firmness of tomato fruit, and postharvest hexanal vapour treatments enhanced firmness of bell peppers (Cheema et al., 2018). In the present study, hexanal vapour exposure for 6 h, 10 h and 16 h significantly protected the fruit texture of bell pepper compared to control (0 h) and 2 h exposure during the entire course of storage. Increased firmness in response to postharvest treatments with hexanal, 1-MCP, and hexanal + 1-MCP was observed in treated cherries, as compared to control after 15 days of storage (Sharma et al., 2010). The loss of pectic substances in the middle lamella of the cell wall is a key step in the ripening process that leads to the loss of cell wall integrity resulting in fruit softening (Solomos and Laties, 1973). In recent studies, it has been demonstrated that in hexanal treated tomatoes, transcript levels of polygalacturonase involved in pectin degradation were down regulated resulting in enhanced firmness and storage quality (Tiwari and Paliyath, 2011). Similar results have also been observed in
apples (Paliyath and Subramanian, 2008), and guava (Gill et al., 2016).

**Fig. 4.4.** Firmness (N) of pepper fruit subjected to 0.01% (w/w) post-harvest hexanal vapour treatments for 0 – 16 h. Fruits were stored for 1, 14, 21 and 28 days in refrigerator incubator at 12°C and 90-95% relative humidity. The data shown are mean ± standard error of three replicates, each containing 3 fruits and letters on the bar indicate significance at p < 0.05, with individual time point analysed separately.

4.4.2.3 Effect of treatments on fruit ripening, physical appearance and shelf life

In order to determine the effect of hexanal vapour treatment time (0 – 16 h) on ripening and shelf life of bell pepper, fruits were visually screened for the presence of infection, spoilage, surface pitting and shriveling during storage for 28 days (Fig. 4.5). Ripening was significantly delayed in peppers treated with hexanal vapour for 6 h, 10 h and 16 h and remained mostly green even after 28 days of storage. Though the peppers exposed to hexanal vapour for 4 h showed some colour development after 28 days of storage, they did not show any sign of damage. By contrast, untreated (0 h) and peppers
treated with hexanal vapour for 2 h gradually developed and were almost ripened by the end of the storage period. Shelf life related qualities of peppers treated for 6 h, 10 h and 16 h were also superior to the control (0 h) and peppers treated for 2 h with respect to colour, firmness and visual appearance. Untreated (0 h) and peppers treated with hexanal vapour for 2 h showed surface pitting and shriveling due to dehydration at the end of the storage period. A deterioration of shelf life related qualities and loss of visual appeal were clearly evident in untreated (0 h) and peppers treated for 2 h by the end of storage period. Based on the results of visual quality, 6 h hexanal vapour treatment time was chosen as the sufficient and most effective exposure time in delaying ripening and enhancing the shelf life of greenhouse bell peppers.

---

**Fig. 4.5.** Appearance of representative samples of pepper fruit on 1, 14, 21 and 28 d of storage. Bell pepper fruit were subjected to 0.01% (w/w) post-harvest hexanal vapour treatments for 0, 2, 4, 6, 10 and 16 h and stored for 28 days at 12°C and 90 – 95% relative humidity.
4.4.3 Effect of hexanal treatment on volatile compounds in bell peppers

Volatile components are important indicators of quality in fruits and vegetables (Buchanan et al., 2000), and have a significant impact on the perception and liking of fruit products by consumers (El Hadi et al., 2013). In the present study, the volatile fractions of untreated bell peppers and fruit treated with hexanal vapour (0.01%) were characterised using headspace solid phase micro-extraction (SPME) gas chromatography after 21 days of storage. The volatile compounds identified in untreated bell peppers are listed in Table 4.3. The change in volatile components in response to hexanal composition is given in Table 4.4. Comparison of the volatile compounds of untreated and treated peppers revealed that a number of volatile compounds are different and only detectable in hexanal treated samples. Overall, the numbers of volatiles in hexanal treated peppers are higher than in the control peppers, results that agree with higher respiration levels reported in our previous studies (Cheema et al., 2018). In other studies, similar results were reported where postharvest hexanal treatments enhanced volatiles in tomato (Tiwari and Paliyath, 2011) and preharvest hexanal treatments increased volatiles in strawberry fruit (Misran et al., 2015). In our current study, the volatile compounds evolved in hexanal treated peppers such as α-ylangene, β-Ocimene, β-phellandrene, d-limonene, 2-octanol, 4-hydroxy-4-methyl, may provide the characteristics of green, fruity and spicy floral scent to peppers; Caryophyllene provides woody, spice and clove scents to pepper fruit. The chromatograms A and B (Fig. 4.6) represent typical chromatograms of the pepper extract representing the peaks of volatile fractions in untreated and hexanal treated fruit respectively. Chromatogram in hexanal treated peppers represented β-Ocimene as the highest peak followed by that of Bicyclo [3.1.1] hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)- and hexanal. In this study, the hexanal formulation treatment had increased the hexanal contents and an additional 11 compounds were identified in treated compared to untreated peppers.
Fig. 4.6. Elution profile of volatiles from the bell pepper fruits as observed during the SPME-GC-MS analyses. (A) untreated fruit and (B) hexanal treated fruit. The identity of individual components is given in Table 4.3 and 4.4 respectively, for the untreated and the treated fruit. Conditions used for the SPME and the analysis are given in the materials and methods (p. 111).
Table 4.3. Volatile compounds of untreated bell peppers stored for 21 days in refrigerator incubator at 12°C and 90-95% relative humidity.

<table>
<thead>
<tr>
<th>Number</th>
<th>Compounds identified in untreated peppers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexanal</td>
</tr>
<tr>
<td>2</td>
<td>1H-Pyrrole, 1-methyl-</td>
</tr>
<tr>
<td>3</td>
<td>1,6-Octadiene, 3-ethoxy-3,7-dimethyl-</td>
</tr>
<tr>
<td>4</td>
<td>D-Limonene</td>
</tr>
<tr>
<td>5</td>
<td>β-Phellandrene</td>
</tr>
<tr>
<td>6</td>
<td>Pentanoic acid, 4-methyl-, ethyl</td>
</tr>
<tr>
<td>7</td>
<td>β-Ocimene</td>
</tr>
<tr>
<td>8</td>
<td>α-Cymene</td>
</tr>
<tr>
<td>9</td>
<td>8-Nonenoic acid, 5,7-dimethylene-, methyl ester</td>
</tr>
<tr>
<td>10</td>
<td>1-Hexanol</td>
</tr>
<tr>
<td>11</td>
<td>2-Octanol, acetate</td>
</tr>
<tr>
<td>12</td>
<td>Nonanal</td>
</tr>
<tr>
<td>13</td>
<td>2-Octanol, (R)-</td>
</tr>
<tr>
<td>14</td>
<td>3-(1,3-Dihydroxyisopropyl)-1,5,8,11,14,17-hexaoxacyclononadecane</td>
</tr>
<tr>
<td>15</td>
<td>Butanoic acid, 2-methyl-, hexyl ester</td>
</tr>
<tr>
<td>16</td>
<td>2,6-Dimethyl-1,3,5,7-octatetraene, E,E-</td>
</tr>
<tr>
<td>17</td>
<td>1-Octanol, 2-methyl-</td>
</tr>
<tr>
<td>18</td>
<td>α-ylangene</td>
</tr>
<tr>
<td>19</td>
<td>trans-α-Bergamotene</td>
</tr>
<tr>
<td>20</td>
<td>Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,1S-(1α,2β,4β)]-</td>
</tr>
<tr>
<td>21</td>
<td>Caryophyllene</td>
</tr>
<tr>
<td>22</td>
<td>α-Bulnesene</td>
</tr>
<tr>
<td>23</td>
<td>Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-</td>
</tr>
<tr>
<td>24</td>
<td>2-Butyl-2,7-octadien-1-ol</td>
</tr>
<tr>
<td>25</td>
<td>α-Guaiene</td>
</tr>
<tr>
<td>26</td>
<td>α-Farnesene</td>
</tr>
<tr>
<td>27</td>
<td>2,5-Octadecadiynoic acid, methyl ester</td>
</tr>
<tr>
<td>28</td>
<td>Hexanoic acid</td>
</tr>
<tr>
<td>29</td>
<td>Pentadecanoic acid</td>
</tr>
</tbody>
</table>
Table 4.4. Volatile compounds of bell peppers treated with hexanal vapour (0.01%) stored for 21 days in refrigerator incubator at 12°C and 90-95% relative humidity.

<table>
<thead>
<tr>
<th>Number</th>
<th>Compounds identified in hexanal treated peppers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexanal</td>
</tr>
<tr>
<td>2</td>
<td>1H-Pyrrole, 1-methyl-</td>
</tr>
<tr>
<td>3</td>
<td>exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol</td>
</tr>
<tr>
<td>4</td>
<td>D-Limonene</td>
</tr>
<tr>
<td>5</td>
<td>β-Phellandrene</td>
</tr>
<tr>
<td>6</td>
<td>8-Nonenoic acid, 5,7-dimethylene-, methyl ester</td>
</tr>
<tr>
<td>7</td>
<td>β-Ocimene</td>
</tr>
<tr>
<td>8</td>
<td>3-Octen-5-yne, 2,7-dimethyl-, (E)-</td>
</tr>
<tr>
<td>9</td>
<td>α-Cymene</td>
</tr>
<tr>
<td>10</td>
<td>1-Hexanol</td>
</tr>
<tr>
<td>11</td>
<td>2-Octanol, acetate</td>
</tr>
<tr>
<td>12</td>
<td>2-Pentanone, 4-hydroxy-4-methyl-</td>
</tr>
<tr>
<td>13</td>
<td>2,4,6-Octatriene, 3,4-dimethyl-</td>
</tr>
<tr>
<td>14</td>
<td>Ethanediolic acid, bis(trimethylsilyl) ester</td>
</tr>
<tr>
<td>15</td>
<td>2,4,6-Octatriene, 3,4-dimethyl-</td>
</tr>
<tr>
<td>16</td>
<td>2-Octanol</td>
</tr>
<tr>
<td>17</td>
<td>Propanoic acid, 2-octyl ester, (R or S)</td>
</tr>
<tr>
<td>18</td>
<td>2,6-Dimethyl-1,3,5,7-octatetraene, E,E-</td>
</tr>
<tr>
<td>19</td>
<td>2,6-Dimethyl-1,3,5,7-octatetraene, E,E-</td>
</tr>
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<td>20</td>
<td>1-Cyclopropene-1-pentanol, α,ε,ε,2-tetramethyl-3-(1-methylene)-</td>
</tr>
<tr>
<td>21</td>
<td>α-ylangene</td>
</tr>
<tr>
<td>22</td>
<td>trans-Sesquisabinene hydrate</td>
</tr>
<tr>
<td>23</td>
<td>trans-Sesquisabinene hydrate</td>
</tr>
<tr>
<td>24</td>
<td>Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-</td>
</tr>
<tr>
<td>25</td>
<td>Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylene)--[1S-1α,2β,4β]-</td>
</tr>
<tr>
<td>26</td>
<td>Caryophyllene</td>
</tr>
<tr>
<td>27</td>
<td>α-Bulnesene</td>
</tr>
<tr>
<td>28</td>
<td>1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1α,4α,4aa,10aa)-</td>
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<td>29</td>
<td>2-Octenal, 2-butyl-</td>
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<td>α-Guaiene</td>
</tr>
<tr>
<td>31</td>
<td>4,5-di-epi-aristolochene</td>
</tr>
<tr>
<td>32</td>
<td>4,5-di-epi-aristolochene</td>
</tr>
<tr>
<td>33</td>
<td>α-Bulnesene</td>
</tr>
<tr>
<td>34</td>
<td>Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-</td>
</tr>
<tr>
<td>35</td>
<td>Methyl 6,8-octadecadiynoate</td>
</tr>
<tr>
<td>36</td>
<td>1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1α,4α,4aa,10aa)-</td>
</tr>
<tr>
<td>37</td>
<td>2,5-Octadecadiynoic acid, methyl ester</td>
</tr>
<tr>
<td>38</td>
<td>Ethanol, 2-(3,3-dimethylbicyclo[2.2.1]hept-2-ylidene)-</td>
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<tr>
<td>39</td>
<td>Hexanoic acid</td>
</tr>
<tr>
<td>40</td>
<td>1-Hexadecanol, 2-methyl-</td>
</tr>
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</table>
4.5 Conclusion

In the present study we demonstrated that hexanal vapour significantly delayed ripening and enhanced the quality of bell peppers. Hexanal vapour applied for 6 h at 0.01% proved the most effective treatment time for enhancing the shelf life and quality of bell peppers. Hexanal concentration in the headspace was reduced rapidly in the presence of pepper fruit over 3 h, which indicated that most of the hexanal is absorbed and metabolized within the tissue. The main volatile compounds were also enhanced in pepper fruit after the treatment. A short exposure to hexanal may thus provide increased quality and shelf life enhancement by limiting membrane deterioration in greenhouse bell peppers.
CHAPTER 5

This chapter is re-formatted from the following publication:

Postharvest hexanal vapor treatment delays ripening and enhances shelf life of greenhouse grown sweet bell pepper (*Capsicum annuum* L.)

Amer Cheema, Priya Padmanabhan, Areeba Amer, Michael J Parry, Loong-Tak Lim, Jayasankar Subramanian, Gopinadhan Paliyath


5.1 Abstract

Aqueous compositions of hexanal, an inhibitor of phospholipase D, has been shown to enhance the shelf life and quality of fruits and vegetables. In the present study, sweet bell pepper fruit were exposed to hexanal vapor and its effect on quality attributes, shelf-life, and antioxidant enzyme activities were evaluated during storage at 7 day intervals for 21 d. Peppers subjected to hexanal vapor treatments (0.005, 0.01, and 0.02%, w/w) showed a significant (p < 0.05) delay in ripening process and preservation of postharvest qualities than untreated peppers, even at 21 d of storage. Treated fruit were characterized by increased firmness, a reduction in physiological water loss and lower electrical conductivity than control fruit, which indicated better membrane preservation. These treatments also resulted in an increase in the levels of antioxidant enzyme activities, specifically that of superoxide dismutase, catalase, glutathione reductase and guaiacol peroxidase. Evidence from the present study indicates that postharvest hexanal vapor treatment at optimal levels can effectively enhance the quality and shelf life of sweet bell peppers.

5.2 Introduction

Bell pepper (*Capsicum annuum* L.) is one of the commercially important horticultural crops grown in greenhouses worldwide and is highly demanded all year round. Bell pepper
fruit is a popular vegetable consumed fresh or used in a variety of processed food products. Peppers are generally considered as a good source of essential vitamins including C, E, A, and B and also rich in many health benefitting antioxidant phytochemicals such as flavonoids, phenolic acids, carotenoids etc., that may reduce the risk of developing chronic degenerative diseases (Marin et al., 2004; Knekt et al., 1996). Bell peppers are currently the object of much attention due to possible links to reduce the risk of life-style related diseases such as arthritis, diabetes and cardiovascular diseases (Lee et al., 1995; Eleyinmi et al., 2002; Nishino et al., 2009; Ozgur et al., 2011). Bell pepper production comprises approximately 500 ha in Canadian greenhouse industry, with an annual production of about 150,000 tons of pepper, worth nearly $500 million CAD (Statistics Canada, 2012). Postharvest losses of sweet bell peppers are estimated at 25–35% of the total production.

Bell pepper fruit are highly perishable and need appropriate handling and adequate care to maintain postharvest quality. The principal physiological factors that negatively impact the postharvest quality of peppers during transportation, storage and marketing are water loss (Lownds et al., 1993) and chilling injury (Hardenburg et al., 1986; Paull, 1990). Currently, there are no targeted practices to prevent these from occurring. The storage life of pepper fruit is also limited by pathological deterioration (Ceponis et al., 1987) caused by Botrytis cinerea and Alternaria alternata (Barkai-Golan, 1981). Botrytis can grow even at the recommended pepper storage temperatures. Refrigerated storage at optimal temperature can help to prolong the shelf life of peppers considerably, though this does not completely prevent the development of fungal infections. Temperatures above the optimal range can encourage postharvest diseases such as soft rot and water loss, while low temperature can often cause chilling injury in bell peppers (Meir et al., 1995). The major postharvest problem with sweet bell peppers is excessive fruit softening that may cause shrinkage, shriveled, drying and pathological disorders which severely reduce the quality and acceptability of the
product by consumers. Pepper fruits deteriorate rapidly during handling and storage due to poor postharvest handling leading to huge losses (Nyanjage et al., 2005).

Maintenance of an efficient antioxidant system can delay the senescence process even though anti-oxidative activity in fruits decreases with aging (Zheng et al., 2007). Stress and senescence enhances the generation of reactive oxygen species (ROS) including superoxide radicals ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ('OH) in various plant cell compartments (Paliyath and Droillard, 1992; Noctor and Foyer, 1998). ROS are lethal and can induce oxidative damage to the cellular components. Plants have developed efficient ROS scavenging systems including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POX), and glutathione reductase (GR) that are able to eliminate ROS (Noctor and Foyer, 1998; Foyer and Noctor, 2011). The antioxidant enzymes including peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and lipoxygenase (LOX) alleviate the potential injury resulting from chilling injury in sweet bell peppers (Wang et al., 2016). The alleviation of chilling injury and maintaining of fruit quality in peppers during cold storage were accomplished through the stimulation of antioxidant enzyme activity and antioxidant gene expression, thus leading to increased protection against oxidative damage to cellular membranes.

Excessive fruit softening resulting in the loss of crispness and firm texture of pepper fruit is a major postharvest concern that can affect consumer preference and market value. Fruit softening is a natural process associated with fruit ripening and occurs due to turgor loss, starch degradation and chemical modifications in the cell wall (Paliyath and Droillard, 1992; Chen et al., 2011). Depolymerization and disintegration of cell wall components particularly pectin by the action of enzymes such as polygalacturanase (PG), pectin methyl
esterase (PME), cellulose and β-galactosidase can further hasten softening (Handa et al., 2007; Ogasawara et al., 2007; Chuni et al., 2010). Studies have shown that activity of the enzyme phospholipase D (PLD) can initiate membrane deterioration during ripening and senescence, and the membrane deterioration is also enhanced by reactive oxygen species (ROS) produced during stress conditions associated with cold storage and postharvest handling (Paliyath and Droillard, 1992). Ways to slow down or reduce the disintegration of cell wall and membrane structures could delay senescence process to some extent. Many biologically active volatile compounds, including hexanal, a natural plant volatile C\textsubscript{6} aldehyde, and an inhibitor of PLD (Paliyath et al., 2003; Tiwari and Paliyath, 2011) with antimicrobial properties have been reported to improve shelf life of fruits and vegetables (Cheema et al., 2014). Hexanal is an FDA approved food additive with GRAS (generally regarded as safe) status and has an ORL-MAM LD\textsubscript{50} of 3.7 g kg\textsuperscript{-1} (EAFUS, 2006). Extensive trials were conducted to study the effect of hexanal/hexanal containing formulations as preharvest sprays, postharvest dips and vapor, on postharvest quality and ripening in a number of fruits and vegetables including sweet cherry, apple, peach, guava, and tomato (Sharma et al., 2010; Cheema et al., 2014; Paliyath et al., 2015; Gill et al., 2016). These experiments were successful in improving the shelf life and visual attributes of produce without impairing fruit color development or compromising the flavour characteristics, while delaying ripening and senescence (Paliyath et al., 2003; Paliyath and Murr, 2007; Misran et al., 2015). One advantage of hexanal is that, being volatile, it can be applied as a vapor with great ease and convenience. The antimicrobial properties of hexanal vapor against major postharvest fungal pathogens in particular \textit{Penicillium expansum}, \textit{Botrytis cinerea}, \textit{Alternaria alternata}, \textit{Sclerotinia sclerotiorum}, \textit{Colletotrichum gloeosporioides}, and \textit{Monilinia fructicola} have been found (Song et al., 1996, 2007; Fan et al., 2006; Thavong et al., 2010; Utto et al., 2008). Previously, hexanal vapor has also been
used to prolong the postharvest quality of longan (Thavong et al., 2010), apple (Lanciotti et al., 1999) and sweet cherry (Sharma et al., 2010).

However, to our knowledge, there is no available scientific literature regarding the effect of hexanal vapour on retaining the quality, shelf life and antioxidant enzymes activity of the sweet bell pepper grown under controlled environment. The principal objective of the present study was to assess the feasibility of postharvest hexanal vapor treatments in extending the postharvest shelf life and quality of bell pepper fruit. The effectiveness of hexanal as vapor application at different concentrations on shelf life and fruit quality of bell peppers were evaluated by analyzing various fruit quality parameters including color, texture, firmness, respiration, weight loss, and the activities of various antioxidant enzymes.

5.3 Materials and methods

5.3.1 Plant material

Sweet bell pepper (Capsicum annuum L. cv. Felicitas) plants were hydroponically grown under natural light in a commercial greenhouse, belonging to Veri Hydroponics Inc., Exeter, Ontario, Canada (Latitude 43° 20 N, longitude 81° 28 W). The plants were grown with a density of 7.4 plants m$^{-2}$ following standard production practices (OMAFRA, 2005). Fruits of uniform size and color at mature green stage were harvested and used for various experiments. The study consisted of two experiments, a small laboratory-scale and a commercial-scale. In both experiments, fruits harvested at 0900 h were transported using cardboard boxes and arrived at the laboratory of University of Guelph the same day at 1100 h. Upon arrival the fruits were sorted and randomly separated into four groups for laboratory-scale and two groups for commercial-scale experiments for performing different treatments.
5.3.2 Post-harvest hexanal vapor treatment of bell pepper fruit

The effect of hexanal vapor treatments on storage life and ripening related-quality parameters of bell pepper fruit was investigated in laboratory-and commercial-scale experimental trials.

**Experiment 1 – laboratory-scale:** Hexanal vapor treatment was carried out according to standard procedure with some modifications (Sharma et al., 2010). Peppers were treated with three different concentrations of hexanal vapor, 0.005%, 0.01% or 0.02% (w/w) and the control fruit were left untreated. Peppers were weighed and placed in 6 mil plastic bags prior to treatment and the bags were closed and sealed immediately (Fig. 5.1) after placing the required amount of hexanal (60 μL kg⁻¹ for 0.005%, 120 μL kg⁻¹ for 0.01% and 240 μL kg⁻¹ for 0.02% respectively) measured into a beaker along with a strip of Whatman 3 filter paper to facilitate fast evaporation (~2 h for complete evaporation). Peppers were exposed to hexanal vapor for 18 h at room temperature. Untreated peppers were also stored for 18 h enclosed in plastic bags under similar conditions. After 18 h of exposure, plastic bags were removed, and thereafter control and treated fruit were stored separately at 12°C and 90–95% relative humidity in refrigerated incubators for 21 d. For weight loss study, peppers were stored separately in containers under the same conditions. Fruit samples were withdrawn at 1, 7, 14 and 21 d of storage for analysis of various quality parameters such as fruit color, firmness, electrical conductivity, and respiration rate as outlined below. Fruit pericarp and flesh tissues were stored at −40°C for antioxidant enzymes assays. Experiment was performed in a completely randomized design having three replications of 3 fruit each per treatment with a total of 180 fruit.
Fig. 5.1. Hexanal vapour treatment of bell pepper fruit

**Experiment 2 – commercial-scale:** In this experiment, fruit were treated with 0.01% (w/w) hexanal vapor as described above and compared with untreated control. Both treatment and control had three replications each and each replicate had 5 kg of peppers. After treatment, peppers were stored in a walk-in cold storage at 12°C with 90–95% relative humidity for 21 d. Samples were withdrawn on 1, 7, 14 and 21 d for quality analysis.

### 5.3.3 Analysis of quality parameters

#### 5.3.3.1 Colour measurements

Pepper fruit colour was recorded by using a Minolta Chromameter (Model CR-300, Ramsay, N.J.) calibrated with a white standard tile ($L = 97.1$, $a^* = 0.29$, and $b^* = 1.82$). For colour measurement, readings were recorded from three points of equator region of each fruit and were expressed according to CIE Lab system. The color was measured on 1, 7, 14 and 21 days after storage in all treated and untreated fruits.
5.3.3.2 Measurement of fruit firmness

Fruit firmness was measured using a penetrometer (Model FT-327, QA Supplies, Norfolk, VA, USA) fitted with a 7 mm diameter stainless steel probe. The penetrometer probe was pushed into the locular space of the whole fruit at basal end of the pepper lobes. The maximum force required to plunge a stainless steel probe was recorded from three lobes of each fruit. The firmness was reported in Newton (N).

5.3.3.3 Measurement of respiration

Representative samples of treated and untreated fruit (3 fruit per container per replicate) were placed separately in 4 L glass bottles at room temperature and the bottles were sealed for an hour. The amount of carbon dioxide in the head space was measured by injecting 3 mL of head-space gas samples into an ADC infrared gas analyzer (Nortech Control Equipment Inc., Etobicoke, ON). The carbon dioxide produced was quantified by comparing to commercial standards.

5.3.3.4 Electrical conductivity/electrolyte leakage

Electrical conductivity (EC) was measured from pericarp discs collected from 3 fruit each per replicate according to standard methods (Ahmed et al., 2010) with some modifications. Discs (10 mm diameter, 5 discs per fruit) were excised from fruit pericarp, washed in distilled water and incubated at room temperature for 2 h in 40 mL of distilled water with an initial 5 min agitation at 60 rpm. EC of the bathing solution was measured using a conductivity meter (Model MW-802, Spectrum Technologies, Inc., IL, USA). EC was calculated on 0.1 kg fresh weight basis and expressed in mmhos cm$^{-1}$. 
5.3.3.5 Measurement of weight loss

Weight loss for each replicate was determined by weighing all samples with sensitive balance (Sartorius ENTRIS6202) at the beginning of the experiment, and then weighed on 1, 7, 14 and 21 days of storage. The difference between initial and final weight of fruits was considered as total weight loss during storage interval and expressed as weight loss percentage (WLP) (AOAC, 2007):

\[
WLP(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]

5.3.3.6 Evaluation of shelf life quality during storage

Samples were removed on 1, 7, 14 and 21 d of storage and fruit were visually evaluated based on overall physical appearance. Fruit were also inspected for the presence of spoilage, microbial infections injury or decay. All the samples were photographed at the end of each evaluation period.

5.3.4 Extraction and analysis of antioxidant enzyme activities

5.3.4.1 Protein extraction

Extracts were prepared from frozen pepper fruit tissues as described by Ahn et al., (2007) with some modifications. Pepper fruit tissues stored at -40°C were weighed (10 g) and homogenized in 10 ml of 100 mM sodium phosphate buffer (pH 7.5) containing 1 mM ethylenediamine tetraacetate (EDTA), 1% polyvinylpyrrolidone (PVP-40) and 1 mM phenylmethylsulfonyl fluoride (PMSF) dissolved in 1 ml dimethyl sulfoxide (DMSO). Homogenization was performed with a Brinkmann homogenizer (Polytron PTA 10) for 2 min. The homogenate was filtered through four layers of cheese cloth and centrifuged (Thermo Scientific Sorvall RC 6 Plus) at 12,000 rpm (28,350g) for 20 min at 4°C. The supernatant
was decanted, and 2 ml of the extract was filtered through a Sephadex G-25 column (GE Healthcare, Sweden) equilibrated with 100 mM sodium phosphate buffer (pH 7.5) at 4°C. The proteins were eluted with 100 mM sodium phosphate buffer (pH 7.5) and stored at -20°C for further analysis. For determining APX activity, all the step in protein extraction were performed as described above, except that the homogenization buffer also contained 5 mM ascorbate. Protein concentration was determined by the Bradford method (Bradford, 1976) with bovine serum albumin (BSA) as standard. The protein content was expressed as μg protein per gram fresh weight.

5.3.4.2 Antioxidant enzymes activity analysis

5.3.4.2.1 Superoxide dismutase (SOD)

The activity of SOD was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) to blue formazan by xanthine oxidase produced superoxide (Vattem et al., 2005; Adyanthaya et al., 2009). The spectrophotometric assay of SOD activity was carried out by monitoring the reduction of NBT at 560 nm (Vattem et al., 2005). A reaction mixture was made containing 13.8 mL of 50 mM potassium phosphate buffer (pH 7.8), 1.33 mM diethylenetriaminepentaacetic acid (DTPA), 0.5 mL of 2.45 Mm NBT, 1.7 mL of 1.8 mM xanthine and 40 IU/mL catalase. To create the reference curve, 0.8 mL of reaction mixture, 100 μL of phosphate buffer, and 100 μL xanthine oxidase were added to a cuvette, and the reaction was followed at 560 nm for 2 min. Using the kinetics mode the amount of xanthine oxidase was adjusted to obtain a linear curve with a slope of 0.3 absorbance per min. The phosphate buffer was then replaced by protein extract and the change in absorbance was monitored at 560 nm for 2 min. One unit of SOD was defined as the amount of protein that inhibits NBT reduction to 50% of the maximum. The activity of SOD is expressed as Units/mg of protein.
5.3.4.2.2 Catalase (CAT)

Catalase (CAT) activity was determined by following the consumption of H$_2$O$_2$ (extinction coefficient, 39.4 mM$^{-1}$ cm$^{-1}$) at 240 nm for 3 min as described by Rao et al. (1996). The reaction mixture contained 100 mM phosphate buffer (pH 7.0), 10 μg equivalent volume of protein extract. The reaction was initiated by adding 10 μL of 10% H$_2$O$_2$ in a final volume of 1 mL. The unit for CAT activity was nanomoles of hydrogen peroxide oxidized per minute per microgram of protein (nmol H$_2$O$_2$ min$^{-1}$ μg$^{-1}$ protein).

5.3.4.2.3 Glutathione reductase (GR)

Glutathione reductase (GR) activity was measured using the method described by Anderson (1985). GR activity was measured by following the oxidation of β-nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. A 2.7 mL assay mixture containing 100 mM phosphate buffer (pH 7.5), 0.1 ml of 0.5 mM glutathione disulfide (GSSG), 0.2 mM NADPH and 100 μg equivalent volume of protein extract was used to assess GR activity. The reaction was initiated by the addition of 40 μL of NADPH. The change in the absorbance of the reaction solution at 340 nm was determined, and the GR activity expressed as nanomoles of NADPH oxidized per minute per microgram of protein (nmol NADPH min$^{-1}$ μg$^{-1}$ protein).

5.3.4.2.4 Guaiacol peroxidase (POX)

The activity of POX was measured by the method of Rao et al. (1996). The enzyme activity was determined by the rate of guaiacol oxidation in the presence of H$_2$O$_2$ (extinction coefficient, 26.6 mM$^{-1}$ cm$^{-1}$) at 470 nm for 4 min. The reaction mixture contained 100 mM potassium phosphate buffer (pH 6.5), 16 mM guaiacol, 10 μg equivalent volume of protein extract, and the reaction was initiated by adding 10 μL of 10% H$_2$O$_2$. The unit for POX
activity was nanomoles of guaiacol oxidized per minute per microgram of protein (nmol guaiacol min\(^{-1}\) µg\(^{-1}\) protein).

5.3.4.2.5 Ascorbate peroxidase (APX)

The assay of ascorbate peroxidase (APX) determination is based on the decrease in ascorbate at 290 nm as ascorbate is oxidized. APX activity was measured by the method of Rao et al. (1996) by determining the ascorbate (molar extinction coefficient, 2.8 mM\(^{-1}\) cm\(^{-1}\)) dependent decomposition of H\(_2\)O\(_2\). The decomposition of H\(_2\)O\(_2\) was measured by following the decrease in A\(_{290}\) for 5 min. The mM reaction volume contained 100 mM potassium phosphate buffer (pH 7.5), 0.5 mM ascorbate, 0.2 mM EDTA and 10 µg equivalent volume of protein extract. The reaction was initiated by adding 10 µL of 10% H\(_2\)O\(_2\). The unit for APX activity was nanomoles of ascorbate oxidized per minute per microgram of protein (nmol ascorbate min\(^{-1}\) µg\(^{-1}\) protein).

5.3.5 Statistical analysis

Analysis of variance was used to determine treatment effect. Where treatments were different means were separated using the Tukey’s test. The general linear models (GLM) procedure of SAS\textsuperscript{®} software (The SAS System, 2002–2010, version 9.3, SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. To compare means of treated fruit with control fruit, a type I error rate P < 0.05 was used for all trials. Linear regression and Pearson’s correlation coefficient (r) of enzyme activities versus quality parameters were calculated.
5.4 Results

5.4.1 Laboratory-scale experiment

5.4.1.1 Effect of hexanal vapour treatments on quality parameters

5.4.1.1.1 Effect of hexanal vapor treatments on ripening, physical appearance and shelf life

In order to determine the effect of hexanal vapor treatments (0.005, 0.01 and 0.02%, w/w) on ripening and shelf life quality, pepper fruit were visually screened for the presence of any infection, spoilage, surface pitting and wilting after 1, 7, 14 and 21 day of storage (Fig. 5.2). As shown in Fig. 5.2, ripening was significantly delayed in hexanal vapor treated peppers, and this delay in ripening was most conspicuous in 0.02% hexanal vapor treated peppers. Peppers in this treatment remained mostly green even after 21 d of storage, but showed some signs of stem wilting and brittleness. Though the peppers exposed to 0.005% hexanal started color development after day 21 of storage, they were also devoid of visual signs of damage. By contrast, untreated peppers gradually developed red colour and were fully ripened by the end of the storage period. Shelf life related qualities of treated peppers were also superior to control fruit with respect to color, firmness and visual appearance. Treated peppers were able to preserve their shelf life qualities, as well as visual appearance during 21 d storage because the peppers showed minimal damage and spoilage due to dehydration and infection. On the contrary, untreated peppers showed wilting and surface pitting due to dehydration at the end of the storage period. A deterioration of shelf life related qualities and the loss of visual appeal were clearly evident in untreated peppers by the end of the storage period. Based on the results of visual quality, 0.01% hexanal vapor treatment was chosen as the most effective treatment in delaying ripening and enhancing the shelf-life of bell peppers.
5.4.1.1.2 Effect of hexanal vapour treatments on fruit colour

Changes in color parameters of bell peppers treated with 0.005, 0.01 and 0.02% hexanal vapor and stored for 21 d at 12°C are shown (Table 5.1). Untreated peppers showed a gradual increase in brightness (L) during the 21 d storage period which increased from 32.38 (day 1) to 37.14 on 21 d, indicating an increase in reflectance due to continuous surface wax biosynthesis during storage. No significant difference in brightness was noticed between control and treated fruit until 14 d of storage. Peppers treated with 0.02% hexanal
vapor showed a decrease in their brightness compared to control and other vapor treatments (0.005% and 0.01% hexanal) on 21 d of storage. In untreated fruit, green color gradually decreased, and the fruit turned completely red by the end of storage period. This is indicated in their $\alpha^*$ values which increased from $-8.40$ on day 1 to $21.66$ by day 21. On the other hand, hexanal vapor treated bell peppers demonstrated a noticeable reduction in red color development (reduced $\alpha^*$ compared to control) during the storage, which in turn may be due to a delay in ripening. This delay in color development was more prominent in fruit treated with the highest hexanal vapor concentration (0.02%). These fruit maintained negative $\alpha^*$ values ($-1.75$) even towards the end of storage period (Table 5.1, Fig. 5.2). The yellow color intensity ($b^*$) did not vary significantly between control and different treatments.

Table 5.1. Effect of hexanal vapor treatments (0, 0.005, 0.01, and 0.02%) on $L$, $a^*$, $b^*$ color coordinates of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Statistically significant ($p < 0.05$) values within the columns are designated by different letters. Lines across the columns showing individual time point analysed separately.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after treatment</th>
<th>Color parameters</th>
<th>$a^*$ (Red)</th>
<th>$b^*$ (Yellow)</th>
<th>$a^<em>/b^</em>$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$L$ (Brightness)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>32.38 ± 0.28b</td>
<td>$-8.40 \pm 0.20a$</td>
<td>10.65 ± 0.27b</td>
<td>$-0.79$</td>
</tr>
<tr>
<td>0.005% Hexanal</td>
<td>1</td>
<td>33.59 ± 0.35a</td>
<td>$-9.85 \pm 0.14b$</td>
<td>12.34 ± 0.24a</td>
<td>$-0.80$</td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>1</td>
<td>33.85 ± 0.36a</td>
<td>$-10.13 \pm 0.18b$</td>
<td>12.51 ± 0.32a</td>
<td>$-0.81$</td>
</tr>
<tr>
<td>0.02% Hexanal</td>
<td>1</td>
<td>33.64 ± 0.44a</td>
<td>$-9.81 \pm 0.21b$</td>
<td>11.92 ± 0.36a</td>
<td>$-0.82$</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>33.49 ± 0.68a</td>
<td>$-3.30 \pm 1.95a$</td>
<td>12.05 ± 0.65a</td>
<td>$-0.27$</td>
</tr>
<tr>
<td>0.005% Hexanal</td>
<td>7</td>
<td>34.75 ± 0.42a</td>
<td>$-7.40 \pm 0.49a$</td>
<td>13.15 ± 0.42a</td>
<td>$-0.56$</td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>7</td>
<td>33.52 ± 0.46a</td>
<td>$-7.98 \pm 0.62a$</td>
<td>12.14 ± 0.35a</td>
<td>$-0.66$</td>
</tr>
<tr>
<td>0.02% Hexanal</td>
<td>7</td>
<td>33.71 ± 0.48a</td>
<td>$-9.17 \pm 0.53a$</td>
<td>12.53 ± 0.34a</td>
<td>$-0.73$</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>36.19 ± 0.76a</td>
<td>$3.58 \pm 2.41a$</td>
<td>14.48 ± 0.64a</td>
<td>0.25</td>
</tr>
<tr>
<td>0.005% Hexanal</td>
<td>14</td>
<td>36.44 ± 0.86a</td>
<td>$-2.31 \pm 1.68ab$</td>
<td>14.58 ± 0.77a</td>
<td>0.16</td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>14</td>
<td>36.34 ± 0.90a</td>
<td>$-1.11 \pm 1.66ab$</td>
<td>14.69 ± 0.73a</td>
<td>0.08</td>
</tr>
<tr>
<td>0.02% Hexanal</td>
<td>14</td>
<td>34.42 ± 0.58a</td>
<td>$-6.73 \pm 1.43b$</td>
<td>13.32 ± 0.55a</td>
<td>0.51</td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>37.14 ± 0.42ab</td>
<td>$21.66 \pm 0.95a$</td>
<td>15.79 ± 0.30ab</td>
<td>1.37</td>
</tr>
<tr>
<td>0.005% Hexanal</td>
<td>21</td>
<td>37.81 ± 0.78a</td>
<td>$0.42 \pm 2.30b$</td>
<td>16.39 ± 0.59a</td>
<td>0.03</td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>21</td>
<td>37.95 ± 0.76a</td>
<td>$0.07 \pm 1.97b$</td>
<td>16.15 ± 0.68a</td>
<td>0.00</td>
</tr>
<tr>
<td>0.02% Hexanal</td>
<td>21</td>
<td>34.88 ± 0.68b</td>
<td>$-1.75 \pm 2.32b$</td>
<td>13.86 ± 0.58b</td>
<td>$-0.13$</td>
</tr>
</tbody>
</table>
5.4.1.3 Effect of hexanal vapour treatments on fruit firmness

A continuous and gradual decline in fruit firmness was observed in control during prolonged storage for 21 d. On the contrary, fruit exposed to different concentrations of hexanal vapour showed significantly enhanced firmness than the untreated fruit at various sampling time throughout the entire storage time (Fig. 5.3). However, no significant differences in firmness were noticed among the various vapor treatments at various time points. Peppers treated with 0.02% hexanal vapor showed the highest firmness of 100.03 N on day 7, 91.80 N on day 14, and 88 N on 21 d after treatment than the other hexanal vapor (0.005 and 0.01%) treated fruit. In 0.01 and 0.02% hexanal vapor treated fruit, an overall increase in firmness of ~27% and 30% was recorded respectively, compared to control. Untreated fruit had 41% reduction in firmness by the end of the storage period as compared to fruit treated with 0.005%, 0.01%, and 0.02% hexanal vapour, which showed a minor (10–15%) loss in firmness (Fig. 5.3).

![Graph showing firmness over time for different vapour treatments](image_url)

**Fig. 5.3.** Effect of hexanal vapor treatments (0.005, 0.01, and 0.02%) on firmness of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Different letters indicate statistical significance (p < 0.05), with individual time point analysed separately.
5.4.1.1.4 Effect of hexanal vapour treatments on electrical conductivity

Electrolyte leakage was determined by measuring the electrical conductivity (EC) of the fruit tissues. The electrical conductivity negatively correlated with fruit firmness. High conductivity is an indicative of leakage of intracellular ions and, therefore, damage to the membranes caused reduction in fruit firmness. The effect of different hexanal vapor treatments on EC of pepper fruit discs was measured and the results showed that EC increased gradually during storage (Fig. 5.4). Control fruit showed significantly higher (p < 0.05) EC values than hexanal vapor treated fruit at all time points from day 1 until day 21. In control fruit, EC on day 1 was 3.46 mmhos cm\(^{-1}\) which was nearly 18% higher than the fruit exposed to 0.02% hexanal on day 1. After 7 d of storage, control fruit showed a 79% increase in EC (6.99 mmhos cm\(^{-1}\)) compared to the EC (3.91 mmhos cm\(^{-1}\)) of fruit treated with 0.02% hexanal vapor. Though EC values did not show significant differences among various hexanal vapor treatments (0.005, 0.01 and 0.02% vapor) until 14 d, marked differences between the lowest vapor treatment i.e. 0.005% and higher levels (0.01 and 0.02% hexanal vapor) were noticeable towards the final stage of storage. In general, higher concentration of hexanal vapor resulted in lower EC values. Control fruit showed significantly higher levels of EC (10.83 mmhos cm\(^{-1}\)), while the fruits treated with 0.01% and 0.02% hexanal showed EC values of 7.11 and 6.41 mmhos cm\(^{-1}\) respectively, after 21 d of storage. At a lower concentration of hexanal (0.005%), the treated fruit showed nearly the same level of EC as shown by the control. These results suggest that hexanal treatment at optimal concentrations reduce membrane damage which is reflected in reduced leakage of ions, and EC (Fig. 5.4).
**Fig. 5.4.** Effect of hexanal vapor treatments (0.005, 0.01, and 0.02%) on electrical conductivity of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Different letters indicate statistical significance (p < 0.05), with individual time point analysed separately.

### 5.4.1.1.5 Effect of hexanal vapour treatments on physiological weight loss

Irrespective of treatment status, the fruits in general exhibited weight loss during their storage period (Fig. 5.5). But weight loss was much more evident in the untreated control compared to the treated peppers. Control fruit showed a significant increase (p < 0.05) in physiological weight loss during 21 d storage, and by the end of the storage period, untreated fruits had lost 10.51% of their initial weight. The lowest weight loss was recorded in peppers treated with 0.01% hexanal vapour throughout the storage period as compared to other vapour treatments. The fruits treated with 0.005% and 0.02% hexanal also maintained lower weight loss than the control (Fig. 5.5). At all sampling time, except on day...
1, weight loss percentage of control was significantly higher than the fruits treated with hexanal vapor.

![Graph showing weight loss percentage over days after treatment with different concentrations of hexanal vapor.]

**Fig. 5.5.** Effect of hexanal vapor treatments (0.005, 0.01, and 0.02%) on weight loss (%) of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Different letters indicate statistical significance (*p* < 0.05), with individual time point analysed separately.

### 5.4.1.1.6 Effect of hexanal vapour treatments on fruit respiration rate

The effect of hexanal vapour treatment on respiration rate in terms of CO₂ evolution of the whole pepper was evaluated. Initially (day 1), CO₂ production of peppers treated with 0.02% hexanal was higher than the control, and peppers treated with 0.01% hexanal (Fig. 5.6). But towards the latter stage of storage especially on 7 d and 14 d, CO₂ production of all the samples decreased sharply and the treatments did not differ significantly from that of the control. However, by the end of storage on 21 d, respiration rate of the peppers treated with 0.02 and 0.01% hexanal increased and carbon dioxide production was significantly higher than the control. The respiratory activity in terms of CO₂ production were 26.7 and 25.9 mL kg⁻¹ h⁻¹ for peppers treated with 0.02 and 0.01% hexanal, respectively (Fig. 5.6).
Fig. 5.6. Effect of hexanal vapor treatments (0.005, 0.01, and 0.02%) on carbon dioxide production of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Different letters indicate statistical significance (p < 0.05), with individual time point analysed separately.

5.4.1.2 Effect of hexanal treatments on protein content and antioxidant enzyme activities

5.4.1.2.1 Effect of hexanal vapour treatments on protein concentration levels

The sweet bell pepper fruits showed various levels of protein content during the storage period. The protein content levels of the pepper fruits treated with hexanal were increased from their initial levels during storage (Fig. 5.7). Initially (day 1), there were no differences in protein concentration levels between treated and untreated peppers. The protein levels of pepper fruits treated with 0.01% and 0.02% (w/w) hexanal were significantly (p<0.05) higher than control fruits on day 7 of storage, but thereafter peppers treated with 0.01% (w/w) hexanal showed significantly higher protein levels than control on day 14 of storage. No major differences in protein levels were found towards the end of
storage period, though the protein levels were slightly higher in hexanal treated peppers than the control. The protein levels of fruits treated with 0.01% (w/w) hexanal increased significantly from 1232 μg g⁻¹fw on day 1 of storage to 1808 μg g⁻¹fw on day 7, but thereafter declined to 1543 and 1227 μg g⁻¹fw on day 14 and 21 respectively. The fruits exposed to 0.01% (w/w) hexanal vapours showed a 47% and 25% higher protein levels on day 7 and 14 respectively from their initial protein levels.

![Fig. 5.7. Effect of hexanal vapor treatments (0, 0.005, 0.01, and 0.02%) on protein concentration of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Different letters indicate statistical significance (p < 0.05), with individual time point analysed separately.](image)

5.4.1.2.2 Effect of hexanal vapour treatments on superoxide dismutase (SOD) activity

In general, higher levels of SOD activity were noticed in treated fruit than untreated control at various sampling time of postharvest storage (Fig. 5.8). A significant (43%) rise in SOD activity was observed in peppers exposed to 0.02% hexanal, compared with control on day 1 of storage. After 7 d of storage, about 134% increase in SOD level (652.5 U) was detected in peppers subjected to 0.02% hexanal vapor treatment compared to control
(279.0 U). After 14 days of storage, 0.01% hexanal treated peppers demonstrated a dramatic increase (804.0 U) in SOD activity than control (489.6 U). At the end of storage period (21 d), fruits treated with 0.02% hexanal showed 71% higher SOD activity (535 U) followed by 68% (526.4 U) and 62% (509.6 U) activity respectively, in 0.005% and 0.01% hexanal treatments, than control (313.7 U).

![Graph](image)

**Fig. 5.8.** Effect of hexanal vapor treatments (0, 0.005, 0.01, and 0.02%) on SOD activities of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Different letters indicate statistical significance (p < 0.05), with individual time point analysed separately.

### 5.4.1.2.3 Effect of hexanal vapour treatments on catalase (CAT) activity

Catalase (CAT) activity was significantly higher in all hexanal vapor treatments than control during the entire storage period (Fig. 5.9). On day 1, a sharp increase in CAT level (more than 8 fold higher compared to control) was noticed in fruit treated with 0.02% hexanal vapor when compared to control and the other vapor treatments. Level of CAT activity also increased steadily in control with respect to storage time, though the enzyme activity was always lower than the different treatments. CAT activity was proportional to
hexanal vapor concentrations; CAT activity of 0.02% hexanal vapor treated peppers remained slightly higher than the other treatments. CAT activity levels in 0.02% hexanal treated fruit reached a maximum value on 7 d, exhibiting values that were 194%, 72% and 28% higher relative to its levels in control, 0.005% and 0.01% hexanal treated fruit respectively. There were increases in CAT activities in 0.005% and 0.01% hexanal treated samples on 14 d, relative to 7 d values. Although CAT levels in treated fruit decreased after 21 d of storage, its level was higher in hexanal treated pepper fruit, relative to control (Fig. 5.9).

![Graph showing CAT activities](image)

**Fig. 5.9.** Effect of hexanal vapor treatments (0, 0.005, 0.01, and 0.02%) on CAT activities of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Different letters indicate statistical significance (p < 0.05), with individual time point analysed separately.

5.4.1.2.4 Effect of hexanal vapour treatments on glutathione reductase (GR) activity

The activity of glutathione reductase (GR) was significantly higher in hexanal treated fruit than the untreated control, except in 0.005% hexanal treatment on day 1 of storage. A
significantly elevated GR level was observed in peppers exposed to 0.01% hexanal compared to control on day 1 of storage (Fig. 5.10). On day 7 of storage, no significant variations in GR activity were detected among the various vapor treatments. GR activity in hexanal vapor treated fruit was enhanced by 27–35% after 14 d of storage compared to untreated control. However, at the end of storage period (21 d), fruit treated with 0.02% hexanal showed nearly 27% higher GR activity than control.

![Graph showing GR activities of pepper fruit under different hexanal vapor treatments](image)

**Fig. 5.10.** Effect of hexanal vapor treatments (0, 0.005, 0.01, and 0.02%) on GR activities of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Different letters indicate statistical significance (p < 0.05), with individual time point analysed separately.

### 5.4.1.2.5 Effect of hexanal vapour treatments on guaiacol peroxidase (POX) activity

The effects of hexanal treatments on guaiacol peroxidase (POX) activity in pepper fruit are depicted in Figure 5.11. All treatments enhanced POX activity, relative to control, during the entire 21 d storage period. POX activity was significantly higher in the 0.01% hexanal vapor-treated fruit (0.103 nmol guaiacol min⁻¹ μg⁻¹ protein) followed by 0.02%
hexanal treated (0.070 nmol guaiacol min⁻¹ μg⁻¹ protein), compared to control (0.041 nmol guaiacol min⁻¹ μg⁻¹ protein) on 7 d of storage. Towards the later stages of storage, particularly on 14 and 21 d, fruit treated with 0.02% hexanal showed significantly higher POX activity than the control. On day 7 of storage, 151% higher POX activity was observed in fruit exposed to 0.01% hexanal, followed by 71% and 34% higher activity in 0.02% and 0.005% hexanal treatments, respectively, relative to control. POX activity in 0.02% hexanal treated fruit reached a maximum level on 14 and 21 d of storage, exhibiting values that were 43% and 38% higher respectively, relative to the activity in control fruit (Fig. 5.11).

![Fig. 5.11. Effect of hexanal vapor treatments (0, 0.005, 0.01, and 0.02%) on POX activities of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Different letters indicate statistical significance (p < 0.05), with individual time point analysed separately.](image)

### 5.4.1.2.6 Effect of hexanal vapour treatments on ascorbate peroxidase (APX) activity

The effect of treatment of fruit on changes in the activity of ascorbate peroxidase (APX) is shown in Figure 5.12. Peppers treated with 0.01% (w/w) hexanal showed
significant differences in APX activity compared to control during the day 1 and 7 of storage. Thereafter, no significant differences were observed in the activity levels of APX between control and hexanal treated peppers. However, hexanal treated fruits showed slightly rising trend in APX activity levels than control on day 14 and 21 of storage.

![Graph showing APX activity levels](image)

**Fig. 5.12.** Effect of hexanal vapor treatments (0, 0.005, 0.01, and 0.02%) on APX activities of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error of three replicates. Different letters indicate statistical significance (p < 0.05), with individual time point analysed separately.

### 5.4.1.2.7 Correlation between changes in enzyme activity changes and fruit quality changes

Pearson's correlation analyses were conducted between changes in activities of enzymes and different fruit quality parameters. The values of the parameters and Pearson's correlation coefficient values are given in Supplementary Tables S5.1 and S5.2, respectively. For correlation analysis, data collected from 21 d stored fruit were used.
general, an increase in enzyme activity was correlated with decreasing parameters such as weight loss and electrical conductivity. There was clear negative correlation between activities of enzymes like catalase and peroxidase, and weight loss as well as electrical conductivity. Though APX and GR activities showed strong negative correlations with electrical conductivity, activities of these enzymes showed slight negative correlation with weight loss.

5.4.2 Commercial-scale experiment

5.4.2.1 Effect of hexanal vapour treatments on quality parameters

5.4.2.1.1 Effect of hexanal vapor treatments on ripening, physical appearance and shelf life

According to the results of small-scale experiments, 0.01% hexanal vapor treatment of peppers helped to enhance the shelf life and preserved the visual attributes of peppers as well. In order to further verify the effectiveness of 0.01% hexanal vapor treatment on storage life of bell peppers, and also to test whether the results obtained in small scale experiments could be reproduced in a larger-scale, separate experiments were conducted in which bell peppers were treated with 0.01% hexanal vapor. After vapor treatments, fruit were stored in a walk-in cold storage at 12°C and samples were removed on 1, 7, 14 and 21 d of storage for measurement of quality parameters. Samples were also visually evaluated to assess their storage quality and ripening progression. As expected, vapor treated fruit showed delayed ripening and delayed color development with respect to untreated fruit. Hexanal treatment also preserved the postharvest qualities of pepper fruit (Fig. 5.13). As shown in Figure 5.13, quality of hexanal treated peppers were superior to control with respect to colour, firmness and visual appearance. By contrast, the control peppers were over-ripened, soft, infected, surface pitting and spoilage had started to set in.
Fig. 5.13. Commercial scale trial to study the effect of 0.01% hexanal vapor treatment on quality parameters of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Appearance of representative samples of bell peppers on day 1, 7, 14 and 21 d of storage. Control refers to untreated peppers.

5.4.2.1.2 Effect of hexanal vapour treatments on fruit colour, firmness, EC and weight loss

Distinct differences in color, firmness, EC, and weight loss were also noticed between control and treated peppers in the large scale trial (Table 5.2 and Table 5.3). Untreated peppers had enhanced brightness, a*, and b* values than the treated peppers, that suggested a delay in color development and ripening during storage. Untreated peppers developed color during storage, as evidenced in the increase in a* values (3.89 on 21 d) compared to treated peppers that still maintained the green color (a* – 7.58 on 21 d). Similarly, treated fruit were also characterized by lower EC, weight loss, and higher firmness values than the untreated fruit during the various sampling time (Table 5.3).
**Table 5.2.** Commercial scale trial to study the effect of 0.01% hexanal vapor treatment on \( L^a \) \( b^a \) color coordinates of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error from three biological replicates of five fruit each. Statistically significant (\( p < 0.05 \)) values within the columns are designated by different letters. Lines across the columns showing individual time point analysed separately.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after treatment</th>
<th>Color coordinates</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( L ) (Brightness)</td>
<td>( a^b ) (Red)</td>
<td>( b^b ) (Yellow)</td>
<td>( a^b/b^b )</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>36.79 ± 0.43a</td>
<td>-10.43 ± 0.22a</td>
<td>14.76 ± 0.30a</td>
<td>-0.71</td>
<td></td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>1</td>
<td>36.50 ± 0.55a</td>
<td>-10.56 ± 0.19a</td>
<td>14.33 ± 0.38a</td>
<td>-0.72</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>37.15 ± 0.44a</td>
<td>-8.45 ± 0.56a</td>
<td>14.82 ± 0.32a</td>
<td>-0.57</td>
<td></td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>7</td>
<td>34.88 ± 0.37a</td>
<td>-9.74 ± 0.13b</td>
<td>12.93 ± 0.28a</td>
<td>-0.75</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>37.78 ± 0.55a</td>
<td>-2.61 ± 1.56a</td>
<td>15.77 ± 0.39a</td>
<td>-0.17</td>
<td></td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>14</td>
<td>34.68 ± 0.45a</td>
<td>-8.35 ± 0.43a</td>
<td>12.98 ± 0.34a</td>
<td>-0.64</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>39.43 ± 0.54a</td>
<td>3.89 ± 1.97a</td>
<td>17.33 ± 0.39a</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>21</td>
<td>36.97 ± 0.53a</td>
<td>-7.58 ± 0.62b</td>
<td>14.46 ± 0.40b</td>
<td>-0.52</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5.3.** Commercial scale trial to study the effect of 0.01% hexanal vapor treatment on quality parameters of pepper fruit stored for 21 d at 12°C and 90–95% relative humidity. Control refers to untreated peppers. The data shown are the mean ± standard error from three biological replicates of five fruit each. Statistically significant (\( p < 0.05 \)) values within the columns are designated by different letters. Lines across the columns showing individual time point analysed separately.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after treatment</th>
<th>Quality parameters</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EC (mmhos cm(^{-1}))</td>
<td>Firmness (N)</td>
<td>Weight loss (%)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>3.80 ± 0.21a</td>
<td>80.76 ± 1.78b</td>
<td>0.47 ± 0.02a</td>
<td></td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>1</td>
<td>3.28 ± 0.12b</td>
<td>101.80 ± 1.85a</td>
<td>0.43 ± 0.01a</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>6.29 ± 0.13a</td>
<td>80.23 ± 2.33b</td>
<td>4.54 ± 0.18a</td>
<td></td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>7</td>
<td>4.36 ± 0.07b</td>
<td>89.24 ± 1.56a</td>
<td>3.02 ± 0.11b</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>7.80 ± 0.11a</td>
<td>68.08 ± 2.07b</td>
<td>7.19 ± 0.29a</td>
<td></td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>14</td>
<td>5.02 ± 0.08b</td>
<td>88.57 ± 1.64a</td>
<td>4.91 ± 0.10b</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>9.51 ± 0.45a</td>
<td>58.77 ± 1.50b</td>
<td>10.37 ± 0.39a</td>
<td></td>
</tr>
<tr>
<td>0.01% Hexanal</td>
<td>21</td>
<td>5.18 ± 0.14b</td>
<td>85.60 ± 1.82a</td>
<td>5.99 ± 0.13b</td>
<td></td>
</tr>
</tbody>
</table>

**5.5. Discussion**

Bell pepper fruit is a globally important vegetable crop for the fresh and processed food market. Pepper fruits have a relatively low storage quality due to their sensitivity to low temperatures, rot development, and water loss. Decay and shriveling to moisture loss are the primary causes of loss among bell peppers postharvest, the susceptibility of the bell pepper to these problems makes them one of the more perishable products during storage.
Maturity level, cultivar, postharvest handling, and storage conditions all affect the shelf-life of bell pepper fruits. The rapid rate of postharvest water loss is the primary factor limiting the shelf-life of bell peppers, additionally it can affect other factors influencing shelf-life (Lownds and Banaras, 1994). The maximum acceptable loss of water from bell peppers is only 5% of the original fresh weight (Kays, 1997). Thus, to extend the shelf life of bell peppers, ways to slow the rate of water loss must be considered. Although the reduced temperatures of refrigerated storage help to reduce the rates of water loss, it is still important to reduce those rates even more to maintain high quality bell pepper fruits. Refrigeration does not completely prevent the development of decay, mostly due to *Botrytis cinerea* and *Alternaria alternate* infection and growth (Meir et al., 1995).

The growing global demand of pepper fruits implies several strategies to increase crop production and fruit quality. Hexanal has been reported as an inhibitor of phospholipase D (PLD) to prolong the shelf-life and quality of produces (Sharma et al., 2010; Tiwari and Paliyath 2011; Gill et al., 2016). The involvement of PLD in membrane catabolism that occurs during ripening of fruits and senescence has also been established (Paliyath et al., 1987; Paliyath and Droillard, 1992; Pinhero et al., 2003). Earlier studies have also shown that natural volatiles may be useful for controlling postharvest decay causing organisms (Utama et al., 2002). It has been reported that hexanal was effective against gray mold on seedless table grapes (Archbold et al., 1999). Fallik et al., (1998) found that hexanal concentrations below 0.5 mol/L stimulated *B. cinerea* mycelial development in vitro, while concentrations above it were inhibitory. Fan et al., (2006) reported that spore viability of *Penicillium expansum* was reduced by 94% following exposure to hexanal vapor of 40 μmol/L for 24 h, compared with 20% at 9 μmol/L. The role of PLD in degradation of membrane phospholipid, and the mechanisms involved in activation of PLD during fruit ripening has been a subject of thorough investigation. Hexanal
has been identified as an effective inhibitor of PLD and is under investigations for the preservation and extension of shelf-life in fruits and vegetables. In present study, we have evaluated the effect of hexanal vapour treatments for their effectiveness in enhancing and preserving the shelf-life and quality of greenhouse bell peppers.

The various chlorophylls contained in the chloroplasts are responsible for the color of green bell peppers (Govindarajan, 1986). The color change that occurs with the ripening of bell pepper fruits is due to a change in pigment content. This change is due mainly to the conversion of chloroplasts to chromoplasts during the ripening process and the subsequent changes in pigment synthesis (Kirk and Juniper, 1966; Camara and Moneger, 1978; Govindarajan, 1986; Matsui and Shibata et al. 1997). During the conversion process chlorophyll begins to disappear and the synthesis of carotenoids increases (Camara and Moneger, 1978). The resulting carotenoid content of the chromoplasts is responsible for the color of ripe bell peppers (Kirk and Juniper, 1966; Govindarajan, 1986). The red colour in ripe bell peppers are derived different components, mainly carotenoids and xanthophylls such as capsanthin, capsorubin, cryptoxanthin, violaxanthin, cryptocapsin, and zeaxanthin (Nagle and Villalon, 1979; Govindarajan, 1985). During the maturation and ripening process of red bell peppers, chlorophylls a and b disappear followed by the increased synthesis of various carotenoids (Curl, 1964; Camara and Moneger, 1978; Govindarajan, 1985). During the early stages of ripening the concentration of lutein decreases rapidly and eventually completely disappears and is replaced by capsolutein (Curl, 1964; Davies and Matthews, 1970; Camara and Moneger, 1978; Govindarajan, 1985).

The hexanal treated fruits had lower (negative) values of $a^+$ (red colour) which indicated that the fruits were remaining green, while $a^+$ values for control gradually increased during the period of storage which reflected fruit ripening. The red component $a^+$ of control fruits increased from -8.40 to 21.66 and from -10.43 to 3.89 in laboratory-scale
and commercial-scale experiments respectively in 21 days. Likewise, this study indicated
that red colour intensity increased with storage time. Gómez-Ladrón de Guevara et al.
(1996) found that all chromaticity values in paprika pepper move from the negative $a^+$
(green component) to the positive $a^+$ values (red component). Similar results were obtained
by Fox et al. (2005) in bell pepper which developed typical red color during storage.
Accumulation of carotenoid pigments is responsible for the characteristic red colour of ripe
peppers (Kirk and Juniper, 1966; Govindarajan, 1986; Fraser et al., 1994; Opiyo and Ying,
2005). On the contrary, hexanal treated tomato fruits developed a full red colour (Tiwari and
Paliyath, 2011). The fruit lightening ($L$) values increased with the storage time. In our
study, the increase of $L$ values in control fruits during fruit ripening probably indicated that fruits
become lighter by changing colour from green to yellow-red as indicated by in the colour
scale for green peppers (Kader and Cantwell, 2010). In contrast to these results, Pérez-
López et al., (2007) found that as luminosity decreases, pepper fruits become darker.
Peppers exposed to hexanal in laboratory-scale trials showed higher $L$ values and reduced
red colour intensity than control fruit on day 21 of storage, suggesting a delay in ripening.
These results are in agreement with earlier studies (Tiwari and Paliyath, 2011) where a
postharvest treatment with EFF formulation enhanced shelf-life of tomato fruit for over 21
days of storage without any damage.

Electrical conductivity (EC) can be used effectively as a physical maturity index, and
it is a suitable index of storage quality (Feng et al., 2005). High EC is indicative of leakage
of intracellular ions and, therefore, damage to membranes. The delay of ripening with
increased firmness was associated with reductions in fruit softening and in electrical
conductivity, the latter being an indicator of membrane permeability (Hershkovitz et al.
2005). In the present studies, the electrical conductivity (EC) of the pepper fruit tissue
gradually increased during storage, suggesting a gradual loss of cell membrane integrity
reflecting the strong negative correlation with fruit firmness. It is evident from the data that electrical conductivity of pepper fruit was influenced significantly with the exposure of hexanal in both laboratory- and commercial-scale trials. Fruits exposed to 0.02% (w/w) hexanal resulted in lowest EC values than those subjected to 0.005% and 0.01% (w/w) hexanal or that of control fruits which is negatively correlated with firmness. These results are in agreement with our earlier studies (Cheema et al., 2014), where a preharvest treatment with 0.01% EFF formulation increased the firmness of tomato fruit by 100% compared to that of untreated tomatoes. These results also support the findings of Sharma et al., (2010) who reported that 1-MCP and hexanal treated cherries showed the highest firmness after 15 days of storage. A linear correlation between turgor pressure and firmness in cherry fruit has also been observed previously (Lustig, 1987; Martinez-Romero et al., 2006). These results are also consistent with that of Cai et al., (2006) who reported a reduction in the firmness of loquat fruit during storage was increased by ethylene treatment and delayed by 1-MCP application. Hershkovitz et al., (2005 and 2009) showed that the delay of ripening of avocado fruit was associated with reductions in fruit softening and in electrical conductivity (EC), the latter being an indicator of membrane permeability. In this context, Sarang et al., (2008) found that the increase in EC is a further indication of increasing senescence of the tissue. (Montoya et al., 1994; Castro et al. 2004 and Feng et al., 2005) added that in avocado fruit, EC has been shown to serve as a good indicator of membrane permeability, and to be highly correlated with ethylene production, softening, and chilling injury symptoms. Thus, electrical conductivity seems to be a suitable index of pepper quality condition or status during cold storage and ripening. Control fruit showed high EC and suffered a complete loss of firmness at the end of storage period that caused excessive softening and shrivelling of fruit. Softening of pepper fruit is caused either by breakdown of insoluble protopectin into soluble pectin or by hydrolysis of starch (Mattoo et
In ripening process, the loss of pectin substances in the middle lamella of cell wall is a key step that leads to the loss of cell wall integrity resulting in fruit softening (Solomos and Laties, 1973). Significantly higher pectin content was recorded throughout storage period for guava fruits treated with 0.015% EFF (Gill et al., 2016). In hexanal treated tomatoes, transcript levels of polygalacturanase and β-galactosidase involved in pectin degradation were down regulated resulting in elevated firmness and enhancing shelf-life (Tiwari and Paliyath, 2011).

Firmness is a critical quality attribute in the consumer acceptability of fresh fruits and vegetables; it is one of the most important quality parameters in pepper which is closely associated with ripeness and shelf-life. Ripening is a complex biochemical process involving several catabolic processes leading to changes in texture and nutritional quality of fruit. Whether consumed green or coloured, high quality bell peppers are those fruits which are firm with a fresh crisp texture and are free of excessive softening, shriveling, bruises, abrasions, and diseases (Ryall and Lipton, 1979; Luning et al., 1994; Sethu and Prabha, 1996; Bosland and Votava, 2000). The texture of bell pepper, and in particular their crispiness, is an important quality attribute to consumers. In pepper fruits, an excessive softening is a major postharvest problem, and ripe fruits are likely become flaccid more quickly than green fruits. Fruit softening is primarily due to changes in cell wall carbohydrate metabolism that result in a decrease of certain structural components of cell wall, mainly pectins (Bartley and Knee, 1982). Pectins can be degraded by the enzymatic reactions which are catalyzed by a combined action of pectic enzymes, mainly pectin methylesterase (PME) and polygalacturonase (PG), which can lead to drastic textural changes of fruits and vegetables (Whitaker, 1984). The enzymatic degradation by PME and PG due to successive demethoxylation and depolymerization reduces intercellular adhesiveness and tissue rigidity (Alonso et al., 1997), and therefore plays an important role in process induced
textural changes. The final degradation products are short and de-esterified pectin chains that cause an increase of pectin solubility, loosening of cell walls, and softening of tissues (Van Buren, 1979) and, therefore, care should be taken after postharvest, during storage and processing. Earlier studies showed that treatments that increase the PME activity would in particular have an effect on improving the texture of fruits and vegetables (Knorr, 1993; Stute et al., 1996). In bell peppers, PME activity can cause de-methylation of pectin molecules in the middle lamella (Alvarez et al., 2001). The de-esterified pectins are consequently less susceptible to β-eliminative degradation and, therefore, more heat resistant and less soluble, which is generally thought to increase the cell-cell adhesion (Ng and Waldron, 1997), and can crosslink with calcium ions, forming calcium pectates that contribute to increase firmness and improving texture (Lee and Howard, 1999). In present study, hexanal vapour treatment to bell pepper significantly protected the fruit texture compared to the control fruits during the entire course of storage. These results might be related to the enhancement of PME activity in the hexanal treated fruits. These results are in accordance with Gill et al., (2016) who observed that EFF (0.015% v/v hexanal) treated guava fruits showed a gradual increase in PME activity during storage, which appears to show a significant influence in determining the postharvest shelflife and quality in guava fruit. In another study, EFF treatment of tomatoes has also been shown to downregulate the transcript levels of PME (Tiwari and Paliyath, 2011). After EFF treatment a decrease in PME transcripts along with a decline in PG transcripts, may lead to a decrease in breakdown of pectin and leading to the enhancement of firmness during storage.

The one of the principal physiological factors that negatively impacts pepper fruit quality during shipment and storage and subsequent marketing is water loss (Smith et al., 2006). The quality of most fruits and vegetables declines drastically with only small weight loss (3% to 10%) and may make them unacceptable for sale (Robinson et al., 1975). The
weight loss rates in peppers are smaller as the size and maturity of the fruit increase (Diaz-Perez et al., 2007). A 5% weight loss in bell pepper is considered to be the maximum acceptable limit (Wills et al., 1998), above which the fruit shows shrivelling and become unmarketable (Mahajan et al., 2009). In our study, a more than 5% weight loss occurred in control fruit; whereas, the fruit treated with hexanal maintained lower weight loss during the entire course of storage and helped in maintaining the marketability of fruit up to 21 days. This is in agreement with previous studies wherein hexanal/hexanal formulation application significantly decreased the weight loss compared to control (Gill et al., 2016; Dek, 2015).

Biochemical changes induced after the application of hexanal may have helped preserve the membrane integrity and cell structure resulting in reduced catabolic processes and quality losses (Tiwari and Paliyath, 2011; Paliyath and Subramanian, 2008). The current observations in pepper are also similar to those reported by Sharma et al., (2010) with hexanal formulation in sweet cherry.

The respiration rate of a product is often a very good indicator of the perishability of a fruit product (Knee, 2002), and it has been demonstrated that respiration is a chemical reaction that involves multiple enzymes in the fruit (Kader and Mitchell, 1989). Studies of respiration and gas exchange focus most closely on carbon dioxide, oxygen, and ethylene. It has been shown that the cuticle of pepper fruit has different permeabilities to oxygen and carbon dioxide. Removal of the cuticle from the fruit eliminates this difference. This side with the theory that pepper fruits conduct the majority of their gas exchange through calyx (Banks and Nicholson, 2000). In our study, it was found that the respiration rate of peppers treated with hexanal was higher than the control. By the 21 day of storage, CO₂ evolution was considerably reduced in control fruits potentially indicating that these fruits are reduced in quality. The results of this study are in agreement with Tiwari and Paliyath (2011) who stated that the CO₂ evolution in hexanal vapour-exposed tomato fruits was higher than in
control fruits even up to 18th day after the treatment, perhaps due to metabolite channelling. A reduction in phospholipase D activity by hexanal treatment has the potential of reducing respiration by reducing the catabolism of free fatty acids liberated during the membrane phospholipid catabolism. However, this may be compensated by channeling more carbon intermediates into the respiratory cycle resulting in increased respiration due to the lowered demand for substrates to replace membrane phospholipids that are broken down by PLD action. In another study, respiratory CO$_2$ production in tomato fruits treated with hexanal was significantly higher than the control throughout the storage period (Dek, 2015).

Antioxidants help to prevent cells by interacting with and stabilising free radicals and prevent some damage they might cause (Hamid et al., 2010). Under stress conditions and senescence in plants, an increase in the generation of reactive oxygen species (ROS) takes place in different cell compartments, including superoxide radicals (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (OH) (Del Rio et al., 1998; Apel and Hirt, 2004; Bhattacharjee, 2005; Foyer and Noctor, 2005; McCarthy et al., 2011). These species can react with proteins, lipids and nucleic acids altering the biological potentiality of these biomolecules (Halliwell and Gutteridge, 2007) and the plants are especially susceptible to the oxidative damage produced by ROS (Gómez et al., 1999; Mittler, 2002; Jiménez et al., 2003). The cellular oxidative damage plays an important role in determining the relative efficiency of the cell functions and, therefore, the behaviour of plants under stress conditions. Cells from higher plants contain antioxidant systems that are able to scavenge ROS, and the cell to cope with various stress situations. An efficient antioxidant system is essential for the maintenance of cellular compartmentalization and preservation of nutritional components and antioxidants (Kalt et al., 1999; Shaham et al., 2003; Hayat et al., 2005; Ahn et al., 2007). The main antioxidant enzymatic systems are catalase (CAT), superoxide dismutase (SOD),
ascorbate peroxidase (APX), glutathione reductase (GR) and guaiacol peroxidase (POX). In the present study, progressive increase in all the antioxidant enzyme activities were observed during the entire storage period in all the treated fruits compared with control samples. The SOD activity declined in control fruits during storage for 21 days, while the peppers exposed to hexanal showed significantly higher SOD activities. Consistent with this observation higher SOD activity in hexanal vapour treated cherry fruits has been reported previously (Sharma et al., 2010). Therefore, enhancement in the antioxidant capacity in response to hexanal vapour treatment provides a beneficial role in enhancing the shelflife of pepper fruits. Moreover, hexanal treatment may enhance the antioxidant potential in peppers resulting in a high reactive oxygen species scavenging potential. In addition, the activities of downstream enzymes such as ascorbate peroxidase (APX) are critical for removal of hydrogen peroxide generated by SOD activity. During storage, pepper fruits exposed to hexanal vapour showed elevated level of APX activities, which is in agreement with previous studies (Sharma et al., 2010). The results of this study are also in the agreement with Ige (2012) who found that antioxidant enzymes activities were higher in green beans treated with hexanal. Progressive increase in CAT activities was also observed during the 21 days of storage in all the treated fruits compared to control fruits. The effective destruction of active oxygen species requires the action of several antioxidant enzymes acting concomitantly with non-enzymatic antioxidants. CAT are important oxyradical detoxification enzymes in plant tissues (Xing et al., 2011). In response to stress, plants normally increase the activity of these enzymes (Xing et al., 2011) and a decrease in antioxidant enzymatic potential may be associated with a reduction in the capacity to prevent damage. Bell peppers are sensitive to freezing injury, which occurs at temperature at or below 0°C (Bosland and Votava, 2000). The freezing point of bell peppers is -0.7°C (Kays, 1997). The symptoms of freeze injury include water-soaked tissue, extreme
softening of tissue, surface pitting, shriveling, and darkened stem and calyx which then become water-soaked. Low temperature storage increased chilling injury of fruits and decreased POX enzyme activities. However, chilling tolerant fruits exhibited lower chilling injury and higher POX enzyme activities (Sala, 1998). In present study, the hexanal treatments enhanced the enzyme activities of POX compared to control, which is in the agreement with previous studies conducted by Ige (2012) who found higher POX enzyme activities in green beans treated with hexanal.

Pearson’s correlation analysis suggest that an increase in antioxidant enzyme activities after hexanal treatment may enhance the protection cellular membranes, thus reducing their damage during ripening and preventing water loss from fruits. This supports the notion that increased membrane preservation may result from exposure to increasing levels of hexanal. Such treatments can be anticipated to have increasing levels of inhibition of phospholipase D, the key enzyme involved in phospholipid degradation and membrane deterioration. A well preserved membrane can prevent loss of water from the tissue, as well as facilitate the continued function of cell wall biosynthesis. Thus, elevation of antioxidant function that occurs as a result of hexanal treatment appears to play a significant role in increasing shelf life and improving quality of bell pepper fruits.

5.6 Conclusion

The present study demonstrated that hexanal applied as vapor has the potential for effectively delaying ripening and preserving the postharvest quality of bell pepper fruit. Hexanal vapor applied at 0.01% was observed as the optimal level for prolonging the shelf life of bell peppers.
CHAPTER 6
General Discussion

The need for food is increasing permanently due to the constantly expanding population, which currently is more than 7.0 billion worldwide (FAO, 2017). Food and Agriculture Organization (FAO) estimates, the current world population is expected to reach 8.5 billion by 2025, and 9.8 billion in 2050. This increase translates into 40% more human mouths to feed, making it the most urgent demand to fulfill. According to Alexandratos and Bruinsma (2012), food supplies would need to increase by 60% in order to meet the food demand in 2050. Food availability and accessibility can be increased by increasing production, improving distribution, and reducing the losses. Thus, reduction of post-harvest food losses is a critical component of ensuring future global food security. Food and Agriculture Organization of U.N. predicts that about 1.3 billion tons of food are globally wasted or lost per year (Gustavsson, et al., 2011). Reduction in these losses would increase the amount of food available for human consumption and enhance global food security. A growing concern is rising food prices due to growing consumer demand, increasing demand for biofuel and other industrial uses, and increased weather variability (Mundial, 2008; Trostle, 2010). In addition to an increase in the proportion of the world’s population that suffers from chronic hunger (prevalence of under nourishment), the number of undernourished people on the planet has also increased to 815 million, up from 777 million in 2015 (FAO, 2017).

Over the past few years, significant focus and resources have been allocated to increase food production. For example, 95% of the research investments during the past 30 years were reported to have focused on increasing productivity and only 5% directed
towards reducing losses (Kader, 2005; Kader and Roller, 2004; WFLO, 2010). Increasing agricultural productivity is critical for ensuring global food security, but this may not be sufficient. Food production is currently being challenged by limited land, water and increased weather variability due to climate change. To sustainably achieve the goals of food security, food availability needs to be also increased through reductions in the post-harvest losses at farm, retail and consumer levels. Food losses do not merely reduce food available for human consumption, but also cause negative externalities to society through costs of waste management, greenhouse gas production, and loss of scarce resources used in their production. Food loss is estimated to be equivalent to 6-10 percent of human-generated greenhouse gas emissions (Gustavasson, et al., 2011; Vermeulen, et al., 2012). A significant contributor of this problem is through methane gas generation in landfills where food waste decomposes anaerobically (Buzby and Hyman, 2012). The US Environmental Protection Agency reports that in the United States about 31 million MT of food waste accounted for 14% of the 2008 solid waste produced in the country (EPA, 2011) costs roughly 1.3 billion dollars to landfill (Schwab, 2010; Buzby and Hyman, 2012). This is a societal cost paid through utilities bills and taxes.

Postharvest losses can be quantitative as measured by decreased weight or volume, or qualitative, such as reduced nutrient value and unwanted changes to taste, color, texture, or cosmetic features of produce (Buzby and Hyman, 2012). In the field, heat from the sun together with cellular respiration heat up the produce and this accumulation of heat is known to reduce its shelf life. When fruits and vegetables are handled carelessly, the internal bruises result in physiological damage as well as breaking the skin or splitting (Kader, 2005). This increases the loss of water and the rate of physiological breakdown in addition to providing avenues for infections leading to decay (Grolleaud, 2002). Quantitative food losses along the chain occur in different forms as a result of spoilage or wastage.
Spoilage is used to describe the losses that occur during harvest, transport, storage, processing and packaging (Burden & Wills, 1989). Qualitative postharvest losses occur when produce suffers loss in edibility, nutritional quality, caloric value and consumer acceptability (Knight et al., 2007). The physiological deterioration is caused by high temperature and low humidity conditions, which increases the deterioration rate. This results in unpalatable flavors and failure in ripening of produce (Kader, 2005). The impact of qualitative losses outweighs the occurrence of the actual physical loss. This is because with quality loss, the farmer tends to suffer direct financial loss, transcended through postharvest operators and marketers. This quality loss then increases the cost of quality compliance systems, which monitor standards in the chain. Qualitative postharvest losses along the value chain have the potential of undermining product trade confidence, which eventually results in downward price pressure.

All through the twentieth century, and especially ever since the advent of the Green Revolution, modern agriculture has been striving to feed the ever-increasing human population through improved technology, relying heavily on tremendous inputs of fertilizers, pesticides and various other agrochemicals. Fruits and vegetables are important components of a healthy diet for a healthy population and represent very important and rich sources of essential vitamins, minerals and dietary fiber. Numerous epidemiological studies indicate the positive effect of regular consumption of antioxidant rich fruits and vegetables against reducing the risks for several diseases such as cardiovascular diseases, cancer, alzheimer’s and macular degeneration (Block et al., 1992; Terry et al., 2001). In Canada, the production of vegetables has increased over the last 60 years. Similarly, global fruit and vegetable production has demonstrated a significant increase over the years, especially in developing countries. Known as high-value crops, fruits and vegetables can generate more income, ensure nutritive and diversified food and enhance livelihood of local communities.
Daily consumption of fruits and vegetables has also the potential to improve health. The World Health Organization estimates that low fruit and vegetable intake contributes to 1.7 million deaths worldwide annually (WHO, 2012). Fruit and vegetable intake levels were found to be below the recommended daily intake of 400 g/person (PROFAV, 2011). With the current ‘5 A day’ message, a large gap still exists between the recommended and actual intake; and many consumers worldwide are not receiving the quantity of fruit and vegetables they should have (Serdula et al., 2004; Krebs-smith et al., 2010). Current data from the Canadian Community Health Survey which measured the number of times participants consumed fruit and vegetable, rather than the actual quantity consumed, reported that only 39.5% Canadians aged ≥12 years consume fruit and vegetables 5 or more times per day (Statistics Canada, 2015).

Horticultural products such as fresh fruits and vegetables are very perishable by nature and susceptible to spoilage due to fungi and bacteria infection. Many are also susceptible to mechanical injuries during postharvest handling and distribution. Once harvested, fruits are removed from their source of water, minerals and sustenance. Fruit tissues continue to respire, using available and stored sugars as well as organic acids leading to rapid senescence. Postharvest quality loss is primarily a function of respiration, onset or progression of ripening (climacteric fruit), water loss (transpiration), enzymatic discoloration of cut surfaces, decay (microbial), senescence and mechanical damage suffered during preparation, shipping, handling and processing (Schlimme and Rooney, 1994; Watada et al., 1996). Fruits destined for fresh-cut processors should be harvested as ripe as possible. This makes it critical that temperature-dependent events related to respiration, water loss, pathological decay and ethylene production be strictly regulated during fruit shipment (or storage). During the climacteric ripening stage of many fruits, there
is a dramatic increase in respiratory CO$_2$ and C$_2$H$_4$ production. Non-climacteric fruit, leafy vegetables, non-fruit vegetables as well as roots and tubers, do not have a surge in C$_2$H$_4$ production and generally have only slightly increased respiration as senescence approaches. However, if severely wounded (e.g. by fresh-cut processing), a significant stress-induced production of CO$_2$ and often times C$_2$H$_4$ occurs (Abeles et al., 1992; Brecht, 1995). Fresh-cut processing increases respiration rates and causes major tissue disruption as enzymes and substrates, normally sequestered within the vacuole, become mixed with other cytoplasmic and nucleic substrates and enzymes. Processing also increases wound-induced C$_2$H$_4$, water activity and surface area per unit volume which, may accelerate water loss and enhance microbial growth since sugars also become readily available (King and Bolin, 1989; Watada and Qi, 1999; Wiley, 1994). These physiological changes may be accompanied by flavor loss, cut surface discoloration, color loss, decay, increased rate of vitamin loss, rapid softening, shrinkage and a shorter storage-life. Increased water activity and mixing of intracellular and intercellular enzymes and substrates may also contribute to flavor and texture changes/loss during and after processing. Therefore, proper temperature management during product preparation and refrigeration throughout distribution and marketing is essential for maintenance of quality. Loss of fruit quality starts with the loss of membrane integrity that generally becomes the lead event during senescence and stress (Paliyath et al., 2008). Phospholipase D (PLD), a phospholipid degrading enzyme present in fruit and vegetables is the key enzyme involved in initiating a cascade of catabolic events that leads to the eventual deterioration of the membrane (Paliyath and Droillard 1992; Yuan et al. 2005, 2006). The post-harvest loss of fruits and vegetables is one of the serious issues to be addressed to bridge the gap between demand and supply thereby nutritional security of the world can be achieved.
Greenhouse vegetables crops have been raised from times immemorial without damaging the environment, but the use of chemicals and synthetic pesticides within the last few years has raised serious doubts about their continuous use. In response to consumers’ preference for natural preservatives over synthetic counterparts, the use of naturally occurring compounds to extend the shelf-life of fresh products has been increasing in the food industry over the past decade. Today, various natural volatile compounds have been used to increase the shelf-life of fresh products (Ko et al., 2012; Kostansek, 2002; Sharma et al., 2010). An approach of delaying the progressive membrane degradation was suggested through hexanal (a primary aldehyde) treatment through its PLD inhibition capability (Paliyath et al., 1999; Paliyath and Subramanian, 2008). It was suggested that inhibition of PLD activity could eventually lead to enhanced membrane stability, and thereby increased longevity of horticultural produce. In the past few years, hexanal based formulations have been found to be effective in enhancing the quality and shelf life of many fruits such as apple, pear, peach, sweet cherry, strawberry and mango (Paliyath et al., 1999; Paliyath and Murr, 2007; Misran et al., 2015; Anusuya et al., 2016) and tomato (Utto et al., 2005, 2008; Cheema et al., 2014). Hexanal, is a naturally occurring compound in plants produced through the lipoxygenase pathway after tissue damage. Exogenous application of hexanal formulation is reported to extend the freshness of fruit by inhibiting the enzyme phospholipase-D, which is involved in fruit deterioration (Paliyath et al., 1999; Paliyath and Murr, 2007). At present, several studies are being conducted to study the ability of hexanal based technologies in enhancing post-harvest characteristics of fruits and vegetables.

In the present thesis, the effect of pre- and postharvest hexanal formulations on the ripening related quality parameters and shelf life of greenhouse tomato during ripening on-plant and postharvest storage were analysed. In addition, the effects of postharvest hexanal vapour treatments on shelf life and quality parameters as well as volatile compounds with
antioxidant activity on bell pepper during laboratory- and commercial scale trials were analysed. The release kinetics of hexanal during vapour application along with absorption and metabolism of hexanal within tissues of pepper fruit were also measured.

Tomatoes are extremely perishable with intense metabolic activities at the ripening stage and have a high demand postharvest handling requirement. Their postharvest losses are mainly due to their high susceptibility towards mechanical injury, physiological deterioration, water loss and microbial decay. Suitable technologies and methods to enhance the postharvest shelf life of this fruit are highly desirable due to the extremely perishable nature of tomatoes. Preharvest spraying applications and postharvest dip treatments of hexanal formulations may provide some improvements on their quality and shelf life. This formulation has been proven to maximize the quality and shelf life of fruits, vegetables and flowers, and it has a great scope for commercial application (Paliyath and Subramanian, 2008). Greenhouse tomatoes are considered a non-determinate crop, therefore, preharvest spray applications will expose fruit at various stages of development to hexanal and other ingredients. Results of this study showed that when hexanal was applied as either a preharvest spray application or a postharvest dip treatment to tomatoes, the treatment greatly affected the tomatoes compared to untreated fruits, in quality parameters and shelf life. The difference in the response of fruits towards hexanal treatment could be related to the difference in phospholipase D content in the fruits. Notably, the treatments had an effect on the quality parameters such as colour, firmness, ascorbic acid, soluble solids, and shelf life of the tomatoes. Changes in the colour intensity and quality are the major parameters to indicate the maturity and quality of fresh tomatoes and development of red colour is considered as an index of maturity. The red colour intensity was reduced in the tomatoes treated with hexanal formulations. Therefore, reduced red
colour intensity in hexanal formulation treated tomatoes is a clear indication that ripening processes were inhibited.

Firmness is one of the most important quality parameters which is closely associated with ripeness and shelf life of the fruit and vegetables. The metabolic events responsible for the textural changes leading to fruit softening during maturation and ripening involve loss in turgor pressure (due to an accumulation of osmotic solutes in the apoplast), degradation and other physiological changes in the composition of membranes, modifications in the symplast/apoplast relations, degradation of starch, and modifications in cell wall structure and dynamics (Paliyath and Droillard, 1992; Chen et al., 2011). It is evident from the results that tomato fruit firmness was significantly increased by the application of hexanal formulations in both pre- and postharvest treatments. Increased firmness in hexanal treated tomato could be related to the down-regulation of several cell wall degrading enzymes such as polygalacturonase, pectin methylesterase, β-galactosidase, β-glucanase and cellulase.

The levels of sugars and organic acids are important factors in determining the taste of tomato fruit, and the relative content of these constituents depends on the activity and the interaction of sugar and acid metabolism. Sugar accumulation during fruit growth and ripening is mainly a matter of carbon import in the form of sucrose from photosynthetic leaves, leading to an increase in total soluble solids. In general, hexanal treatments resulted in significantly higher soluble solid contents in the tomato fruit. Pre- and postharvest treatments with hexanal did not result in major changes in pH and organic acid. This reduction of the acidity associated with postharvest ripening has been attributed to the fact that organic acids are substrates for the respiratory metabolism in detached products. In addition, increased ascorbic acid and pathogen resistance have also been observed in tomatoes as a result of hexanal treatments.
Postharvest life and quality of bell peppers are greatly influenced by membrane deterioration and cell wall degradation, and associated changes including dehydration, decrease in firmness, chilling injury and susceptibility to fungal infections, which makes them one of the most perishable products during storage. Bell peppers did not show a response to hexanal dip treatments, and hence, the effectiveness of hexanal vapour treatment was investigated. On application, hexanal evaporated and reached maximum concentration levels within 20-30 min after initiation of the treatment. When incubated in the presence of fruit, hexanal vapour is also being absorbed by the fruit tissues. We have observed that head space hexanal vapour level comes down to 5% of its peak hexanal concentration within 3 h of application in the presence of bell peppers. The levels of metabolites of hexanal, such as hexanol and hexanoic acid were none to minimal before the containers were opened. Therefore, most of the hexanal is absorbed and metabolized within the tissues. Thus, a short exposure to hexanal vapour appears to be adequate for enhancing shelf life and quality of the produce. Evidence from this study clearly demonstrated that hexanal vapour treatment of bell peppers was effective in delaying ripening and preserving the postharvest qualities of fruit. Consumers generally evaluate produce quality by visual assessment. Physical attributes such as fruit colour, shape, gloss, absence of blemishes and infections gain consumer’s attention. Bell peppers exposed to hexanal vapor presented better visual appeal than untreated fruit. Reduced red color intensity and enhanced firmness which are clear indicators of delayed ripening were evident in hexanal vapour treated peppers. Ripening inhibition, as monitored by the delay in red color development, increased when the concentration of hexanal applied was also increased. These observations are similar to earlier studies in which greenhouse tomato fruit were subjected to preharvest hexanal spray, and postharvest dip treatments (Cheema et al., 2014). These treatments reduced red color and enhanced firmness of tomatoes.
suggestive of a delay in ripening and senescence during their storage. Results showed that hexanal vapour application to bell pepper significantly protected the fruit texture even after 21 days of storage. Cell wall and cell membrane play critical roles in preserving the quality and visual appeal of detached fruits. The fruit surface acts as protective barrier preventing water loss, dehydration and leakage of solutes. The delay in ripening and preservation of fruit quality observed in response to hexanal vapor treatments could be ascribed to the direct effect of hexanal on preservation of cell structures which could eventually lead to reduction of water loss that is linked to many of the postharvest related problems. Protection of cell wall and cell structures can also contribute to the reduced incidence of postharvest fungal diseases, which is another principal cause of postharvest decay and degeneration in peppers. The antifungal properties of hexanal are observable at much higher concentrations than used in the present trials, and thus the preservation of shelf life of peppers observed in this study are likely the result from biochemical and molecular alterations induced in the fruit as a result of hexanal treatment.

Electrical conductivity (EC) is an indicator of membrane permeability and storage quality. A gradual increase in EC of the pepper fruit tissue was noticed during storage, indicating a gradual loss of cell membrane integrity. Hexanal vapour treatments resulted in lower EC values in the fruit tissues which is indicative of arresting senescence, decreased membrane deterioration, loss of ionic compartmentation and water loss in the tissues. A rapid rate of water loss is one of the principal physiological factors that negatively impacts pepper fruit quality during shipment, storage and subsequent marketing. Measures to slow down the rate of water loss must be considered to extend the shelf life of peppers. The fruit treated with hexanal maintained a significantly lower weight loss and better external appearance without shriveling during the entire course of storage.
The cellular respiration rate often indicates the perishability of a fruit product. The respiration rate was higher during storage in the peppers treated with the hexanal vapour formulation. A reduction in PLD activity by hexanal treatment has the potential of reducing the catabolism of free fatty acids liberated during the membrane phospholipid catabolism. However, this may be compensated by channeling more carbon intermediates into the respiratory cycle resulting in increased respiration due to the lowered demand for substrates to replace membrane phospholipids that are broken down by PLD action. The increase in respiration may thus be a temporally delayed indication of increased metabolism, as a prelude to ripening induced changes. Though 0.02% hexanal vapor treatment was most successful in delaying the color development and ripening of bell peppers among the 3 different concentrations examined (0.005, 0.01 and 0.02%); 0.01% hexanal vapor was selected as the most effective treatment for preservation of postharvest qualities of peppers. Peppers exposed to 0.02% hexanal vapor developed stem end wilting, which reduced the visual quality during storage. Peppers subjected to 0.02% hexanal vapor also demonstrated higher weight loss percentage and respiration rate than peppers subjected to 0.01% hexanal. Taken together, these results indicate that 0.01% hexanal provided the best headspace hexanal vapor level, and was the most favourable hexanal vapor treatment for preservation of peppers. The potential of hexanal vapor (0.01%) application in preserving the postharvest life of peppers was verified and confirmed again in a large scale experiment that generated observations in agreement with our small scale experiments.

During senescence and stress conditions in plants, an increase in the generation of reactive oxygen species (ROS), including superoxide radicals (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (OH$^-$) occurs in different cell compartments. Antioxidant and ROS scavenging systems in plants effectively help to protect from free radicles and ROS-
induced oxidative damage by interacting with and stabilizing free radicals. The main components of ROS system include antioxidant enzymes such as CAT, SOD, ascorbate peroxidase, GR, and POX, and the antioxidant compounds such as carotenoids, ascorbate, glutathione etc. During storage, pepper fruit exposed to hexanal vapour showed elevated levels of SOD, CAT, POX, GR and APX activities. Both CAT and POX are involved in the direct removal of $\text{H}_2\text{O}_2$. These results suggest that enhancement in the antioxidant capacity in response to hexanal treatment might be resulting in a higher ROS scavenging potential in bell pepper. The results suggest that the antioxidant and ROS system are more active in hexanal treated peppers than in the untreated peppers during their storage and this might have contributed to the preservation of fruit qualities.

Overall, the studies presented in this thesis provide advancement in our understanding of the phospholipase D inhibition technologies for enhancing shelf life and quality of tomato and bell peppers, along with the development of novel approaches to minimize postharvest losses of produce grown in commercial greenhouse. Future work may focus on the molecular approach to investigate the biochemical properties of PLD for better understanding of the involvement of this enzyme in the pathways that lead to membrane deterioration. Future studies are needed to investigate the use of hexanal formulations on other greenhouse products. The potential of hexanal treatment to enhance the quality of greenhouse produce should be investigated through controlled release of hexanal in packaging systems using nanotechnology on a commercial basis.
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APPENDICES

Supplementary Table S5.1. Changes in antioxidant enzyme activities, electrical conductivity and weight loss of pepper fruit stored for 21 days at 12°C and 90 – 95% relative humidity.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Treatments</th>
<th>Enzyme activity (% change)</th>
<th>Electrical Conductivity (% change)</th>
<th>Weight Loss (% Change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>Control</td>
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<td>100</td>
<td>100</td>
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<tr>
<td></td>
<td>0.005%</td>
<td>156.2</td>
<td>80</td>
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<td>159</td>
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<td>100</td>
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</table>
**Supplementary Table S5.2.** Linear regression and Pearson correlation coefficient (r) of enzyme activities versus electrical conductivity and weight loss of pepper fruit subjected to hexanal vapour treatment stored for 21 days at 12°C and 90 – 95% relative humidity.

<table>
<thead>
<tr>
<th>Correlation parameter</th>
<th>Regression equation</th>
<th>$R^2$</th>
<th>Pearson correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD vs. Weight loss</td>
<td>$y = -6.9121x + 164.49$</td>
<td>$R^2 = 0.13$</td>
<td>-0.37</td>
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<tr>
<td>SOD vs. Electrical conductivity</td>
<td>$y = -1.3545x + 244.8$</td>
<td>$R^2 = 0.772$</td>
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<tr>
<td>APX vs. Weight loss</td>
<td>$y = -0.0758x + 101.86$</td>
<td>$R^2 = 0.001$</td>
<td>-0.031</td>
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<tr>
<td>APX vs. Electrical conductivity</td>
<td>$y = -0.1988x + 118.93$</td>
<td>$R^2 = 0.865$</td>
<td>-0.93</td>
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<tr>
<td>CAT vs. Weight loss</td>
<td>$y = -112.82x + 1286.5$</td>
<td>$R^2 = 0.9585$</td>
<td>-0.98</td>
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<tr>
<td>CAT vs. Electrical conductivity</td>
<td>$y = -3.1538x + 401.62$</td>
<td>$R^2 = 0.696$</td>
<td>-0.83</td>
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<tr>
<td>GR vs. Weight loss</td>
<td>$y = 0.0612x + 98.011$</td>
<td>$R^2 = 6e-05$</td>
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<td>GR vs. Electrical conductivity</td>
<td>$y = -0.5805x + 159.67$</td>
<td>$R^2 = 0.895$</td>
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<td>POX vs. Weight loss</td>
<td>$y = -9.6523x + 198.65$</td>
<td>$R^2 = 0.32$</td>
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<tr>
<td>POX vs. Electrical conductivity</td>
<td>$y = -0.9434x + 190.55$</td>
<td>$R^2 = 0.913$</td>
<td>-0.96</td>
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</table>