Role of Intermittent Warming in Reducing Chilling Injury in Tomato

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Abstract

Tomatoes (*Solanum lycopersicum* L.) are an important crop commercially and nutritionally. Tomatoes are often harvested at the mature-green stage and handled at low temperature to facilitate postharvest storage. However, long term storage at low temperature (below 13 °C) is challenging as mature-green tomatoes are susceptible to chilling injury (CI). Chilling injury, therefore, limits the advantage of using low temperature to maintain quality of fresh tomatoes during long term storage.

Failure to develop red colour, uneven blotchy red colouration, excessive softening, and increased susceptibility to decay were found to be main CI symptoms. Different low temperature ranges affected tomatoes differentially. For a given storage duration, storage at 8 °C delayed but did not perturb red colour development, fruit held at 6 °C showed uneven blotchy red colouration and those at 2.5 °C showed a complete failure to develop red colouration and severe decay. It was suggested that there was a series of critical temperature thresholds at which different CI symptoms were induced.

An increased rate of electrolyte leakage is often considered as an indicator of chilling damage to cell membranes. This can be confused with ripening-related increase in electrolyte leakage. This study indicated that electrolyte leakage in mature-green tomatoes increased with chilling either in stored discs or fresh discs cut from stored fruit independently of ripening during long term storage.

Interruption of low temperature storage with one or more short periods of warm temperature for various periods of time (intermittent warming, IW) has been shown to reduce CI and improve keeping quality of several horticultural crops. While IW was effective in reducing CI, the responses were highly dependent on production conditions and cultivar and different symptoms of CI had independent responses to IW. The present study was undertaken to elucidate a basic mechanism (physiological response) by which IW reduces CI in tomato. IW stimulated ethylene production and reduced CI. It was suggested that IW-stimulated ethylene was required to reduce CI in tomato as also reported in some other climacteric fruit. However, blocking ethylene response of IW fruit by 1-methylcyclopropene (1-MCP) reduced chilling-induced decay indicating that reduction of CI symptoms by IW was not solely attributable to ethylene action. IW possibly has some metabolic benefit independent of ethylene.

As adoption of IW in commercial situation is logistically challenging, one objective of the current study was to determine if intermittent ethylene (IE) supply during low temperature storage could be used as an alternative to IW for alleviating CI in tomato. Results suggested that IE supply was effective in reducing CI, although effectiveness was dependent on storage temperature and nature of CI symptoms. Additionally, treating tomatoes with 1-MCP in the absence of IW enhanced decay susceptibility, consistent with ethylene involvement in reducing decay during cool storage. While 1-MCP was found to reduce CI and extend storage life in many crops, our results indicated that 1-MCP may not be considered for commercial use in mature-green tomatoes before cool storage.

Overall, the positive results of ethylene application may assist the tomato industry to store tomatoes for longer periods at chilling temperatures and hence enable sea freight of tomatoes to new markets. While ethylene showed promise as a tool to reduce CI and allow fruit to develop red colour after cool storage, further research is required to determine optimum concentration, time and frequency of application, and efficiency when applied at a range of temperatures in order to derive a successful treatment that may have significant commercial applications. Additionally, while the findings are positive for a possible industry application, the magnitude of the positive effect in reducing CI needs to be determined for other tomato cultivars and fruit from other growing locations. More importantly, if the recommendation of IE application is commercially adopted, in future it will be important to investigate the effect of IE on sensory perceptions of tomato quality.

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"In God we trust, the rest must come with data."

Narayana Murthy, Infosys Chairman

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1. Introduction

1.1. Background

Tomatoes (*Solanum lycopersicum* L.) are one of the most popular and economically important crops in the world (Madhavi and Salunkhe, 1998). Present world production of fresh tomatoes is about 151 million tons produced from 4.4 million hectares with a world trade value of around US$ 6 billion (FAO, 2012). Tomato is also an important crop in New Zealand both nutritionally and economically. Tomato and tomato-based products are used daily in New Zealand cuisine as they are considered to be beneficial for a healthy diet because they have low fat, are cholesterol-free and a rich source of vitamins and antioxidants. The fresh tomato industry in New Zealand has a 'farm gate' value of approximately NZ$80 million and a retail value approaching NZ$115 million. However, tomatoes traded in New Zealand are mostly for domestic consumption. About 5% of the total fresh tomato production, with an annual FOB value of NZ$15.4 million, is exported predominantly to Australia (during summer), and the Pacific Islands (FreshFacts, 2011).

Commercially, tomatoes are harvested at the mature-green stage (i.e., physiologically mature, but pre-ripe) of development and are handled at low temperatures to facilitate shipping (Chomchalow et al., 2002). However, long term low temperature storage of mature-green tomatoes is currently risky because of the likely development of chilling injury (CI). Chilling injury, therefore, limits the advantage of using low temperature to maintain quality of fresh tomato and ultimately restricts flexibility of trade. In New Zealand, export of tomatoes is limited to expensive air freight. Apart from other marketing reasons, the industry does not have a consistent commercial solution for exporting mature-green tomatoes to distant markets by sea freight. It would be possible to export New Zealand-grown tomatoes by sea to Australia or to emerging Asian markets if a potential solution exists to maintain tomato quality around 4 weeks of cool storage and thereafter 3-5 d at supermarket shelf. If 6 weeks of storage is possible, markets in Europe become available.

Low temperature storage is the most effective means of minimising metabolism, hence maintaining quality and extending the storage life of many horticultural products (Saltveit and Morris, 1990) and allowing them to survive long distance transport (Malacrida et al.,

2006). In particular, in a country like New Zealand that is geographically far away from its main export markets, the fresh fruit and vegetables have to travel long distances before they reach to consumers. If handlers of fresh horticultural commodities are able to store products for long periods at low temperature, it can avoid gluts in the market and allow the supply of products at times of shortage. Unfortunately, low-temperature storage can be more detrimental than beneficial for some crops, particularly those of tropical or subtropical origin (Wang, 1990). Equally, if these chilling-sensitive commodities are not refrigerated, they tend to deteriorate rapidly and have a short storage life. This dilemma results in substantial postharvest losses for many horticultural crops (Hardenburg et al., 1986). Additionally, globalisation means products are being exported for long distance shipping and stored for long periods so that the potential for chilling-induced losses is much greater than for air freight products stored for shorter periods.

Postharvest losses resulting from chilling injury are probably greater than has been recognized or quantified (Wang, 1994). The most difficult part of CI studies is that symptoms may not be apparent while the produce is still in cold storage and the symptoms often appear later once the produce has been transferred to ambient temperatures and in the market place. Therefore, a substantial portion of losses resulting from CI may be mistakenly attributed to pathogen-induced or ripening disorders at the market (Wang, 1994).

Alleviation of CI is an important objective in postharvest research. Different postharvest technologies have been suggested to assist in delaying or inhibiting CI. Among the different postharvest means reported to prevent CI problem, intermittent warming is one strategy with potential to reduce CI, yet it is difficult to achieve commercial success or to explore its full potential because of logistical difficulties of warming and cooling large volumes of fruit in a commercial environment.

To reduce losses arising from CI, it is important to understand the biological and environmental factors involved in this physiological disorder. In this chapter, factors involved in inducing CI in many horticultural crops are reviewed with a special focus on tomato. Then, alleviation of CI by different postharvest technologies including IW is briefly summarised. Finally possible modes of action by which IW may reduce CI are discussed in detail.

1.2. Chilling injury of fruit and vegetables

Chilling injury is defined as a physiological dysfunction or abnormality in crops, particularly those of tropical and subtropical origin, that occurs when they are exposed to low, non-freezing temperature (≤ 12 °C) for a period of time (Saltveit and Morris, 1990). Although temperate crops, in general, can cope with low temperature better than those of tropical or subtropical origin, some are also injured if exposed to low temperature for an extended period (Bramlage and Meir, 1990). Chilling injury may occur at different stages in the supply chain including in transit or market distribution, in retail or home refrigerators, even in the field during growth and development (Skog, 1998).

The degree of physical and physiological damage caused by CI depends on the chilling temperature to which the crop is exposed, duration of exposure and sensitivity of the species to that low temperature (Saltveit and Morris, 1990). Crops are generally able to recover from brief exposures to chilling temperature and function normally once low temperature stress is removed. However, if exposure of sensitive species to low temperature (below a threshold level) persists for too long, it causes metabolic dysfunction, and irreversible manifestation of visible symptoms (Lyons, 1973). Rate of development and magnitude of the visible symptoms of injury depend, to a large extent, on tissue type, variety, metabolic status (active or dormant) of the tissue at the time that chilling stress is imposed and on a variety of environmental factors (Bramlage and Meir, 1990).

Development of CI may occur during exposure to low temperature, but the symptoms usually appear after transfer of a product to a warmer, non-chilling temperature (Cheng and Shewfelt, 1988). Some CI symptoms are qualitative in nature, including developmental or metabolic disorders such as incomplete or inhibited ripening, excessive or inhibited softening and deficient aroma and flavour. Physiological symptoms become apparent in different ways including skin depression (pitting), abnormal skin yellowing, tissue decomposition, internal or surface browning, woolly or dry pulp texture, and fungal or bacterial rot (Saltveit and Morris, 1990). Some disorders may affect the skin of the produce but leave the underlying flesh intact; others affect only certain areas of the flesh or the core region (Wills et al., 2007). CI symptoms are sometimes characteristic of a particular crop such as woolly peaches and nectarines, sunken patches on citrus, superficial scald of apple and pear or abnormal colour development of tomatoes.

1.2.1. Possible mechanism of inducing chilling injury

Many theories have been proposed to explain the primary mechanism of CI and subsequent development of its symptoms (Saltveit and Morris, 1990). While CI has been recognised, described, and studied for over 100 years (Molisch, 1896; 1897 as cited in Lyons, 1973), the exact mechanisms of this disorder and its effects are not completely understood (Sevillano et al., 2009; Vega-García et al., 2010; Luengwilai et al., 2012; Sanchez-Bel et al., 2012). Given all the variables of inducing CI, the variety of horticultural crops and their diversity in morphological structure, composition, and developmental stage, understanding of the CI problem is not simple and it is no surprise that numerous reports have been published in an attempt to understand and to clarify this phenomenon.

Cell membrane damage and disruption of membrane integrity under low temperature stress is thought to be the primary cause of CI (Wang et al., 2005). The original hypothesis was proposed by Lyons (1973). The proposed 'membrane theory' postulated that lipid phase transition in cell membrane during chilling exposure constitutes the primary response of this physiological disorder and ultimately causes the molecular and structural alteration in the lipid matrix. The sustained primary damage then leads to a cascade of secondary events that are reflected in tissue damage, including metabolic dysfunction, ionic imbalances, altered metabolism, reduced photosynthesis, accumulation of toxic compounds, altered enzyme activities, and the loss of membrane integrity that leads to visible symptoms of chilling injury (Raison and Orr, 1990). The resulting symptoms are loss of turgor, ion/water leakage, altered ripening, pitting and loss of product quality such as flavour loss (Marangoni et al., 1996; Valdenegro et al., 2005). This concept is illustrated in Figure 1.1.

Therefore, CI is subdivided into two events (Figure 1.1). A temperature dependent primary event is initiated (virtually immediately) when the temperature falls below a threshold temperature for a specified duration, resulting in some metabolic dysfunction. The secondary event is time-dependent and leads to the development of characteristic CI symptoms for a particular crop as a consequence of primary event.

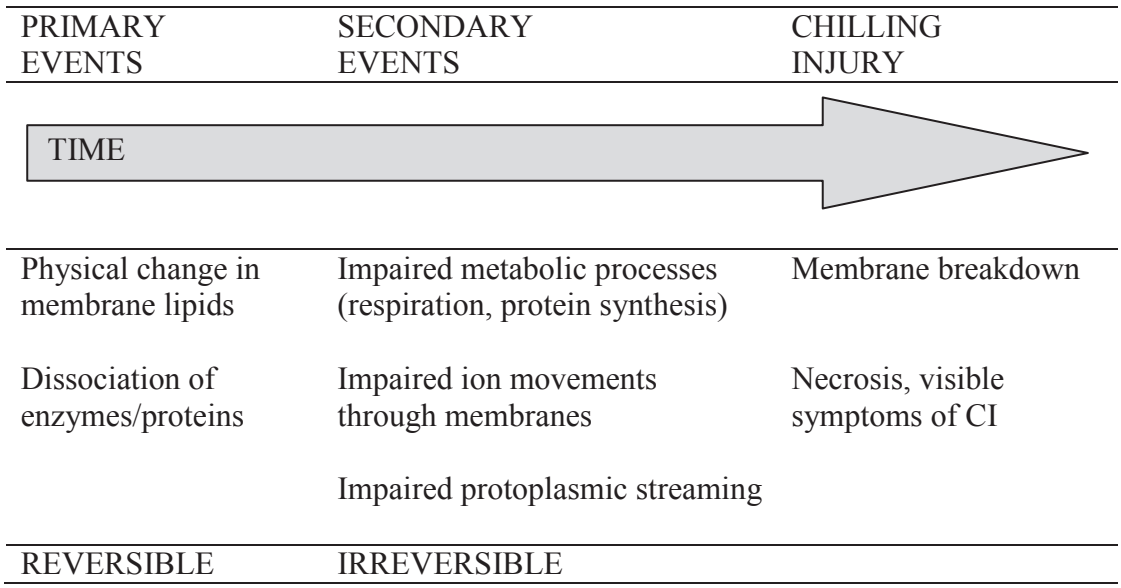

Figure 1.1 Time sequence of events leading to CI (G. R. Chaplin in Wills et al., 2007)

1.2.1.1. Role of lipid composition in cell membrane

Plasma membranes consist of about 80% lipids and proteins, the remaining 20% being carbohydrates. Among the various lipids, phospholipids, glycolipids and sterols are most abundant in the plant cell membranes. However, cellular membranes of different organelles have different lipid-lipid and lipid-protein interactions (Murata and Nishida, 1990). The plasma membrane and tonoplast are rich in sterols, sphingolipids and phospholipids, whereas the mitochondrial and endoplasmic reticulum membranes contain only phospholipids (Murata and Nishida, 1990).

Phospholipids, the most abundant membrane lipids, contain a charged hydrophilic head group that interacts with the external aqueous environment, and two hydrophobic fatty acid tails that interact with each other. These lipid molecules associate with proteins form a bilayer that is fluid in normal physiological conditions above chilling temperatures (Staehelin and Newcomb, 2000). The membrane fluidity in a tissue is greatly affected by the fatty acid composition of the phospholipids and interactions with other membrane components (Staehelin and Newcomb, 2000). Different factors affect biochemical modification of membrane lipid unsaturation and phase behaviour including i) molecular packing ii) dynamic effects iii) effect of chain length and double bond position and iv) hindered motion of hydrocarbon chains (Quinn et al., 1989).

The fluidity of the cell membrane helps it to function normally, and is thought to be important for its resistance and adaptation to various physiological stresses (Lurie et al., 1995). The Lyons (1973) theory supports the idea that with a changing environment such as exposure of chilling sensitive crops to low temperatures, there is a general increase in the microviscosity of the lipid matrix due to a reduction of random rotation or flip-flop of phospholipids and decreased mobility of unsaturated fatty acids. This event results in the fluid membrane transitioning to a semi-crystalline or solid gel state (Raison and Orr, 1990). The phase transition is affected by the nature of the lipids and the ionic strength of cell contents and both gel and liquid phases can coexist (Freedman, 1981). The rigid gel structure may cause loss of membrane elasticity and dysfunction of membrane proteins (Sevillano et al., 2009). Eventually, this may lead to cell membrane rupture, membranes become leaky to electrolytes and diminishing ion gradients across the membranes that are essential for physiological activities of the cell (Murata and Nishida, 1990).

The hypothesis relating chilling sensitivity to a phase transition in membrane lipids have many critical shortcomings (Raison and Orr, 1990). Detailed reviews about the shortcomings of this model can be found in other studies (Parkin et al., 1989; Raison and Orr, 1990; Saltveit, 2002). Other events proposed as influencing the primary cause of CI include i) the redistribution of cellular calcium (Minorsky, 1985) ii) a conformational change in some key regulatory proteins (enzymes) (Graham et al., 1979) iii) a marked decrease in the rate of cyclosis (protoplasmic streaming) and a change in cell cytoskeletal structure (Woods et al., 1984) iv) an accumulation of reactive oxygen species (ROS) resulting in oxidative damage (lipid peroxidation, protein degradation, and DNA damage) leading to membrane breakdown and eventual visible signs of symptoms (Parkin and Kuo, 1989).

Lipid compositions of mitochondrial membranes is also affected during low temperature stress that cause disorders in mitochondrial respiration resulting in loss of metabolic energy of affected cells (Sevillano et al., 2009). Moline (1976) correlated low temperature injury with ultrastructural modifications of fruit organelles including swollen mitochondria and plastids. Lyons and Raison (1970) proposed a consistent incidence of phase transition in the mitochondrial membrane as the result of the physical effect of temperature. However, O'Neil and Leopold (1982) suggested that no bulk phase transition was detectable in mitochondrial membranes of chilling sensitive soybean seed in the temperature range

where chilling occurs and argued that chilling injury is not induced by phase transition. Raison and Orr (1986), however, refuted that argument by observing a phase transition at 15 °C for mitochondria from soybean hypocotyls, at 16 °C for tomato, at 15 °C for cucumber and reinstated that sensitivity to chilling injury is related to a temperature-induced alteration in the structure of cell membranes.

The reduced flexibility of mitochondrial membranes from sensitive tissues below a critical temperature leads to a loss of membrane integrity following phase transition and altered oxidative rate. This depresses the rate of mitochondrial oxidation that could lead to an accumulation of metabolic intermediates or reduced ATP supply and ultimately be responsible for decoupling of oxidative phosphorylation. Mitochondria would then exhibit altered respiration rate (Lyons and Raison, 1970), decreased cytochrome c oxidase activity and enhanced alternative oxidase activity (Prasad et al., 1994). However, these effects are not seen if the cold period is relatively short - only a slight decrease in respiration is observed in this case, but in general oxidative phosphorylation is not affected (Lyons and Raison, 1970). The metabolic dysfunction and disintegration of respiratory activity result in generation of ROS leading to oxidative stress (Shewfelt and del Rosario, 2000). Finally, loss of cell membrane integrity causes cell rupture, cell autolysis and ultimately cell death (Parkin and Kuo, 1989).

A transitory burst of respiration is usually observed specifically following transfer of fruit to non-chilling temperature after being exposed to low temperature for a certain period of time (Lyons and Briedenbach, 1990). An increase in respiration burst at warmer temperature after a chilling period could be attributed to greater accumulation of oxidizable intermediates (Eaks, 1980). Moreover, this increase may be a result of the need for more energy to repair cellular damage from chilling (Luengwilai et al., 2012).

1.2.1.2. Role of ethylene in inducing CI or its prevention

1.2.1.2.1. Ethylene biosynthesis pathway

Ethylene (C_2H_4) is a simple gaseous hormone in plants. The biosynthetic pathway of ethylene production is related with ripening, senescence, storage conditions and biotic and abiotic stresses (Watkins and Ekman, 2004; Lin et al., 2009). Ethylene biosynthesis (Figure 1.2) is usually completed in three major steps - i) Methionine, an amino acid, serves as a

precursor of ethylene in higher plants (Yang and Hoffman, 1984). The formation of SAM (S-adenosyl methionine) from methionine is catalysed by SAM synthetase at the expense of one molecule of ATP per molecule of SAM synthesized. ii) The rate-limiting step of ethylene synthesis is the conversion of SAM to ACC (1-aminocyclopropane 1-carboxylic acid) by ACC synthase (ACS) (Kende, 1993). iii) In the presence of oxygen, ACC is then converted to ethylene by ACC oxidase (ACO). ACS and ACO are encoded by multigene families, with tomato possessing at least nine ACS (*LeACS1A*, *LeACS1B*, and *LeACS2-8*) and six ACO (*LeACO1-6*) genes (Barry and Giovannoni, 2007; Lin et al., 2009).

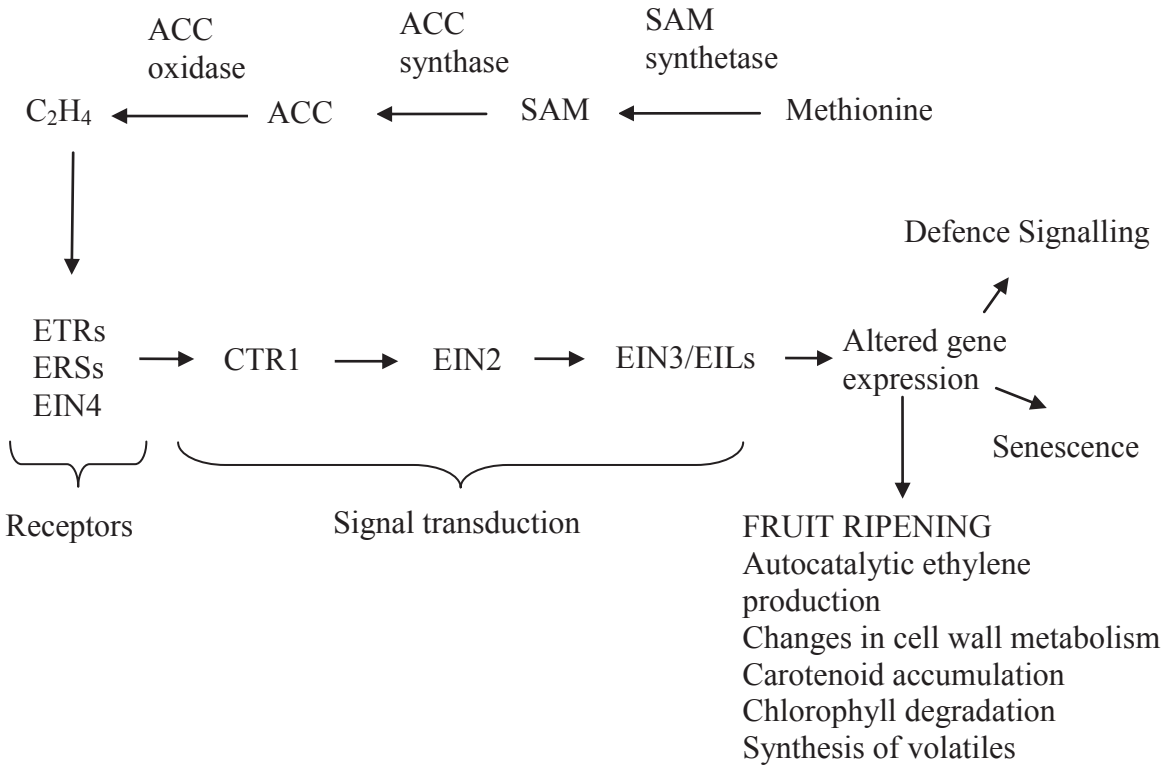

Figure 1.2 Ethylene biosynthetic pathway and signal transduction (Adapted from Alexander and Grierson, 2002).

Ethylene is then perceived by a group of membrane-located receptor proteins including ETR1 (ETHYLENE RESPONSE 1), ERS1 (ETHYLENE RESPONSESENSOR 1), ETR2 (ETHYLENE RESPONSE 2), ERS2 (ETHYLENE RESPONSE SENSOR 2), and EIN4 (ETHYLENE INSENSITIVE 4) (Bleecker et al., 1988; Hua and Meyerowitz, 1998). A chemical signal is then sent to the cell and the ethylene molecule releases. A series of genes are involved in ethylene signal transduction pathway (Figure 1.2). The ethylene

signal is transduced by ethylene insensitive (EIN3) through a pathway involving constitutive triple response (CTR1) and ethylene insensitive (EIN2). The membrane-integrated protein EIN2 (localised in endoplasmic reticulum membrane) is an essential transducer of the ethylene signal, as its loss-of-function mutant displays little response to exogenous ethylene (Alonso et al., 1999). EIN3, a plant-specific transcriptional factor and EIN3-like 1 (EIL1), a homolog of EIN3, play a crucial role in regulation of expression of ethylene responsive genes (Chao et al., 1997; Alonso et al., 2003; Binder et al., 2007). In the nucleus, both transcription factors (EIN3 and EIL1) are necessary and sufficient for the activation of ethylene regulated gene expression and diverse responses (Chao et al., 1997; Alonso et al., 2003). The ethylene signal is transmitted via the action of EIN2 to stabilize EIN3/EIL1, probably by promoting EBF1/2 (EIN3 binding F-box protein 1/2) proteins (An et al., 2010).

1.2.1.2.2. Chilling injury and role of ethylene

Low temperature storage usually reduces ethylene biosynthesis in fruit by reducing ACC concentrations and sharply decreasing activity of the enzyme ACO that becomes irreversible if the chilling period is too long (Etani and Yoshida, 1987; Cabrera and Saltveit, 1990). Lower ACO activities were found in chilled mung beans stored at 0 °C compared to 26 °C (Etani and Yoshida, 1987). Similarly, decreased ACO activity and subsequent low production of ethylene was reported during cold storage of bean leaves and cucumber at 11 and 12 °C respectively (Wang and Adams, 1982). However, ethylene production was increased by addition of ACC to discs of cucumber leaves stored at 5 °C (Wang and Adams, 1982). This suggests that ACC is the limiting factor in ethylene production during cool storage (Hoffman and Yang, 1980).

Upon removal of chilled tissue to warmer temperatures, a strong stimulation of ethylene biosynthesis was observed in many chilling sensitive species (Woolf et al., 1997). Cool storage promoted accumulation of ACS and ACO mRNA that triggered the ethylene burst observed after removal from cool storage (Watkins et al., 1990). The post-chilling rise in ethylene production was usually higher than typical basal or wound ethylene production (Field, 1981). This ethylene burst is a sign of cellular damage and was often correlated with the appearance of CI symptoms (Sfakiotakis and Dilley, 1974; Wang and Adams, 1980) as reported in mango, pear, zucchini, orange and grapefruit (Wang et al., 1985;

Schirra, 1993; Lederman et al., 1997; Balandran-Quintana et al., 2003; Lafuente et al., 2003). Lin et al. (1993), however, questioned the physiological significance of enhanced ethylene production in CI development. They argued that elevated ethylene production could be used to indicate that chilling sensitive tissues had been exposed to chilling temperature but this rise in ethylene production did not necessarily signify its involvement in CI symptom development. Luengwilai and Beckles (2010) suggested that the post-chilling burst in ethylene production was not essential for initiation of CI in tomato. In general, it was proposed that exposure of chilling sensitive species to conditions below or at the critical chilling temperature affected the activity of membrane-bound enzymes, increasing their activation energy and thus determining ethylene production (Field, 1981). Since cell membrane damage has long been considered the primary site of injury and as ethylene receptors are predominantly localized in the endoplasmic reticulum membrane (Chen et al., 2002), changes in cell membrane physical properties could affect ethylene perception (Rugkong et al., 2011). However, it remains unclear whether chilling-induced ethylene promotes the physiological and biochemical changes associated with chilling damage or if it participates in the mechanism of adaptation to chilling stress and/or subsequent defence against damage.

No consistent relationship was found between ethylene production and CI severity between species (Watkins, 2002). Most notably, during low temperature storage ethylene production could either be stimulated or inhibited depending on the species (Watkins and Ekman, 2004). For instance, low temperatures induced ethylene production in apple (Knee et al., 1983; Larrigaudiere et al., 1997) and citrus (Cooper et al., 1969; McDonald et al., 1985) but inhibited the synthesis of ACC and ethylene production in plum (Larrigaudiere et al., 2009). Nevertheless, ethylene production might be stimulated once the chilled fruit were transferred to a reconditioning temperature (Cheng and Shewfelt, 1988; Sanchez-Bel et al., 2012); the magnitude of ethylene peak began to decline as the time of chilling was prolonged (Rugkong et al., 2011). A decrease in ethylene stimulation by a longer chilling period was previously reported in cucumber (Wang and Adams, 1980), possibly as a result of chilling damage to the system that converts ACC to ethylene (Cheng and Shewfelt, 1988). In mango, after long-term cool storage (4 weeks), no significant capacity to convert added ACC to ethylene was observed upon removal to shelf-life conditions (Lederman et al., 1997). Tomatoes stored at 3 °C for 1 week showed an increased ethylene production but the production decreased when fruit were stored for 4 weeks and subsequently

transferred to 20 °C (Rugkong et al., 2011). They found that increased ethylene production in fruit stored for 1 week was associated with either increased or unchanged *ACS2*, *ACS4* and *ACO1* gene expression while lower ethylene production in fruit stored for 4 weeks was associated with reduced *ACS2*, *ACS4* and *ACO1* gene expression. Results for the expression of genes involved in ethylene signal transduction showed different responses to chilling. For instance, expression of the *NR* (nonripening) receptor gene was markedly reduced by chilling that may have delayed ripening (Tieman et al., 2000) while *LeETR1* expression was induced and the expression of *LeETR4* receptor gene was slightly affected by chilling (Rugkong et al., 2011).

Whether the rise or fall in ethylene production from chilling is caused by or is a consequence of, or may play a role in reducing the development of CI, is not well understood (Concellón, 2005). Increased ethylene production may be a mere response to low temperature stress in chilling-sensitive species (Sevillano et al., 2009) or it could be associated with ripening, and it is difficult to separate ripening and stress-related ethylene production (Watkins, 2002). Importantly, it is unclear whether induced ethylene after subsequent warming in higher temperature acts as a defence mechanism directly to cope with chilling stress or indirectly helps fruit to advance in ripening and thus reduces chilling sensitivity.

Although there is no clear-cut interpretation of the significance of chilling-induced ethylene stimulation, chilling sensitivity of tissues can be altered by application of exogenous ethylene. Ethylene application reduced CI in some crops (Ben-Amor et al., 1999), or induced CI in others (Lafuente et al., 2001). For example, ethylene markedly enhanced CI symptoms in 'Shamouti' oranges as indicated by de-greening and decay development (Porat et al., 1999). Avocado fruit stored below 12 °C with ethylene suffered severe tissue discolouration compared with fruit stored in air without ethylene (Lee and Young, 1984). Likewise, blocking ethylene action by 1-methylcyclopropene (1-MCP, SmartFresh^{SM}) in avocados stored at 5 °C caused a reduction in mesocarp discolouration, decay development (Pesis et al., 2002). However, ethylene is not involved in external skin discolouration (Pesis et al., 2002). Exogenous ethylene application accelerated CI symptoms in climacteric plum and inhibition of ethylene action by 1-MCP or nitric oxide reduced CI in plum (Candan et al., 2008; Singh et al., 2009). Equally, removal of ethylene from the atmosphere reduced the incidence of core browning of apple, although the

beneficial effect was cultivar specific (DeEll et al., 2007). 1-MCP reduced CI in climacteric fruit such as persimmons (Salvador et al., 2004) and in non-climacteric fruit such as pineapples (Selvarajah et al., 2001). Furthermore, inhibition of autocatalytic ethylene production by antisense ACC oxidase RNA led to reduced chilling-induced pitting and browning of the rind of cantaloupe melon (Ben-Amor et al., 1999). Together these results indicate that chilling sensitivity of these fruit increases with exposure to ethylene.

In some tissues, however, ethylene has been reported to alleviate CI. For example, treating green tomatoes stored at 2.5 °C with 100 $\mu L.L^{-1}$ ethylene before storage prevented CI and increased marketable life (Chomchalow et al., 2002). However, the authors found that the same treatment after cool storage resulted in delayed ripening without a prolonged marketable life as seen for the pre-storage ethylene treatment. Fresh-cut red-ripe tomato slices in packages with high ethylene concentrations stored at 5 °C had lower CI (indicated by water-soaked areas) than slices stored in packages with low ethylene concentrations (Hong and Gross, 2000), although ethylene treated tomato slices had an undesirable effect of accelerated softening (Pangaribuan, 2009). In contrast, Jeong et al. (2004) indicated that inhibition of ethylene action by 1-MCP reduced water-soaking incidence in tomato slices stored at 5 °C and they suggested that this was an ethylene-mediated symptom of senescence and not a CI symptom as proposed by Hong and Gross (2000). Ogura et al. (1976) did not find any effect of ethylene on CI symptoms when mature-green tomatoes were treated with 50 $\mu L.L^{-1}$ ethylene before storage at 4 °C where no ripening was observed during storage. 'Honeydew' muskmelons treated with ethylene and stored subsequently at 2.5 °C for 15-19 d ripened more rapidly than did untreated melons and showed a reduction of CI (Lipton and Aharoni, 1979). Duration of ethylene exposure was important as 18 h was found more beneficial than 24 h (Lipton, 1980). Bananas treated with propylene (an ethylene analogue) prior to storage at 7 °C showed increased tolerance to CI (Wang et al., 2006) and fruit treated with 1-MCP had increased CI symptoms compared to controls (Jiang et al., 2004). Similarly, inhibition of ethylene action by using 1-MCP induced a greater incidence of woolliness in peaches (Zhou et al., 2001) and nectarines (Dong et al., 2001) by preventing normal ripening. Reducing cold-induced ethylene production by inhibiting ACS (by AVG – Aminoethoxyvinyl-glycine) and ACO (by $CoCl_2$ - Cobalt chloride) enhanced peel damage in 'Fortune' mandarin (Lafuente et al., 2001). These results support the role of ethylene in reducing CI but do not indicate whether

this positive effect is independent of simple ethylene induced ripening and corresponding reduction of chilling sensitivity.

Overall, results reported here indicate that the role of ethylene in development of CI is complex as ethylene treatment can reduce, increase or have no effect at all on CI development. Nonetheless, a reduction in CI symptoms could be due to a slower decline in ethylene production than its inhibition as suggested in non-climacteric pineapple treated with 1-MCP (Selvarajah et al., 2001). It is possible that beneficial or detrimental effects of ethylene depends on whether ethylene increases or decreases product sensitivity to chilling (Watkins and Ekman, 2004). Fruit maturity, species or tissue type within the same species will determine the ethylene responses in altering chilling sensitivity along with ethylene application dose or timing. More importantly, ethylene may influence chilling-sensitivity of tissues but not necessarily increase or reduce all types of symptoms in a given tissue. As chilling injury is a collective term for a set of physiological disorders found in chilling-sensitive tissues, it is possible that the effects of ethylene are not limited to one factor, but could influence one or many symptoms at the same time. Exposure of mature-green tomatoes to ethylene or ethephon before storage at 0 or 5 °C reduced abnormal ripening but not the severity of other chilling symptoms during post-storage ripening at 20 °C (Kader and Morris, 1975). In peaches, application of ethylene during cool storage reduced woolliness significantly but encouraged fruit decay and loss of pulp firmness (Girardi et al., 2005). Exposure of sweet potatoes to ethylene for 2 d at 20 °C following storage at 1 °C for 2-6 d reduced CI severity as indicated by “hardcore” in the roots but ethylene had an adverse effect on flavour and colour (Buescher, 1977). Therefore, it is important to indicate whether induction or reduction of particular chilling symptoms is ethylene dependent or not.

In summary, manifestation of chilling injury is a function of exposure time by temperature. In short term storage, injury processes are reversible if the chilling stress is removed. However, if stress is maintained, the ionic imbalances and/or loss of cellular integrity become excessive and the process becomes irreversible. Afterwards removing the stress or warming to non-chilling temperature only exacerbates injury symptoms (Raison and Lyons, 1986). By separating CI into two events, it becomes possible to differentiate the primary ‘cause’ (i.e. the initial event happening upon chilling) from the secondary ‘effect’ (i.e. the subsequent events that produce physiological and visual signs of CI). It helps to

delineate the fundamental molecular mechanisms underlying this phenomenon, which are enormously complex (Luengwilai et al., 2012).

1.3. Chilling injury symptoms in tomatoes

Chilling injury in tomatoes is common after low temperature storage between 0 and 13 °C for 2 weeks or longer (Efiuvwevwere and Thorne, 1988). Chilling-induced quality changes in tomatoes vary with cultivar (Abou-Aziz et al., 1974), duration of storage (Hobson, 1981), and fruit maturity (Autio and Bramlage, 1986). Generally, mature-green tomatoes are more sensitive to low temperature than ripe fruit (Hobson, 1981) and sensitivity is reduced as fruit approach to full ripeness (Abou-Aziz et al., 1974). A storage temperature considered safe for mature-green tomatoes is 13 °C, whereas 7-10 °C is the threshold temperature for ripe tomatoes (Suslow and Cantwell, 1997).

In literature, inability to ripen and uneven blotchy red colouration, unusual texture (mealiness), pitting, shrivel, and water soaking have been reported as gross CI symptoms in tomatoes (Hobson, 1987; Efiuvwevwere and Thorne, 1988; Jackman et al., 1992). Loss of aroma and flavour is a subtle chilling damage that occurs during low temperature storage and is important in terms of loss of quality (Maul et al., 2000). Increased susceptibility to fungal and bacterial rots (particularly *Alternaria* at the stem scars and as numerous small spots over the fruit surface) is also frequently reported (Thorne and Segurajauregui, 1982). Chill-injured fruit often exhibit increased rates of respiration and ethylene production associated with abnormal metabolism (Cheng and Shewfelt, 1988; Saltveit and Morris, 1990). Additionally, an increased rate of solute leakage in tissues is often correlated with the appearance of CI symptoms (Saltveit, 2002) and measurement of CI severity in tomato (Cabrera and Saltveit, 1990; Luengwilai et al., 2012).

1.3.1. Uneven blotchy colouration and failure to develop full red colour

Consumers often associate fruit colour with flavour, safety, storage time, nutrition, and level of satisfaction (Pedreschi et al., 2006). However, this judgment of quality by fruit colour may not necessarily be right for many fruit and vegetables and can be misleading indicator of quality (e.g. refrigerated supermarket red tomatoes with lack of flavour) (Shewfelt, 2002). Tomatoes harvested for fresh consumption are often picked at mature-green or early ripe stages and transported to retailers at low temperature (Chomchalow et

al., 2002). Depending on temperature uneven blotchy red colouration or complete failure of red colour development in tomatoes can be induced (Cheng and Shewfelt, 1988; Lurie et al., 1996). For instance, at 2 °C, one of the most obvious changes in mature-green tomatoes is complete failure to ripen (Hobson, 1987). Fruit locules remain green and seeds turn brown (Moline, 1976). Storage at 2 or 6 °C can inhibit full red colour development in tomatoes harvested even at advanced maturity stages (i.e. breaker or pink) (Ilic and Fallik, 2005; Gómez et al., 2009).

Tomato colour changes from green to red during normal ripening as chloroplasts transform into chromoplasts, chlorophyll degrades and lycopene, a major carotenoid responsible for red colour development, accumulates (Shewfelt, 2002). During low temperature conditions modification of red colour development may be because chloroplasts are the first organelles that undergo structural changes (Marangoni et al., 1989; Yang et al., 2009). Ultrastructural observations indicated that failure to ripen was due, in part, to interruption in conversion of chloroplasts to chromoplasts while non-chilled fruit showed lycopene crystals in healthy plastids (Moline, 1976). Rugkong et al. (2011) suggested that loss of chlorophyll in tomato during cool storage was manifested as yellowing. Decreased levels of chlorophyll in chilled tomatoes probably unveil β-carotene, causing the appearance of yellow blush colour (Dodds et al., 1991). Chilling may also have caused accumulation of chalco-naringenin, a yellow compound found in tomato pericarp (Baker et al., 1982 as cited in Dodds et al., 1991).

The physiological abnormalities associated with blotchy red colouration could be due to abnormal functioning of random patches of tissues. It is believed that CI is not translocatable (Saltveit and Morris, 1990). Eaks and Morris (1957) found that CI symptoms in cucumber were localised to the half of an intact fruit which was exposed to chilling. Equally, failure of uniform heating may induce differences in CI and colour development between heated and non-heated halves of a tomato (Lu et al., 2010) or an avocado (Woolf, 1997), indicating a localised rather than systemic effect of heat treatment on postharvest quality parameters of tomatoes (Lu et al., 2010). It is possible that uneven red colouration in tomatoes is due to localised failure of patches of tissues to develop red colour. Alternatively, it may be possible that blotchy red colouration is not random rather it is a patterned disruption of normal ripening prototype. Tomato fruit is composed of distinct tissue types including pericarp, placenta, septa and locular gel tissues (Brecht, 1987).

Locular gel develops prior to ripening of the pericarp (Kader and Morris, 1976). Ripening initiates in mature-green tomatoes in locular gel, proceeds through placenta to the columella, with the first visible sign of red (yellow or orange) pigmentation at the blossom end and colour then progresses towards stem end of the fruit (Yahia and Brecht, 2012). A climacteric rise in ethylene production has also been observed in gel tissue prior to other tissue types (Brecht, 1987). It is possible that chilling affects tissues differentially resulted in different ripening behaviour of various tissues and eventually uneven blotchy red colour appears. Additionally, Jiang et al. (1999) observed that 1-MCP treated bananas showed uneven skin de-greening and the authors attributed this to positional differences in the rate of new synthesis of ethylene binding sites. This may also be true for tomato.

Molecular studies reveal that the interruption of gene expression involved in colour development results in altered colour development in chilled tomatoes. Lycopene is an intermediate of the carotene biosynthetic pathway in tomato (Figure 1.3). Carotenoid formation utilizes the ubiquitous isoprenoid precursor geranylgeranyl pyrophosphate (GGPP). Two molecules of GGPP are condensed to form phytoene and this reaction is catalysed by phytoene synthase (PSY). Phytoene is then converted to lycopene in a series of dehydrogenation reactions, which introduce four double bonds into the phytoene molecule. This conversion is performed by the sequential action of phytoene and ζ-carotene desaturase enzymes respectively. β-carotene and α-carotene are then synthesised by the action of the enzyme sesquiterpene cyclase (Alexander and Grierson, 2002). As fruit ripen, the concentration and activity of sesquiterpene cyclase are reduced; leading to the accumulation of lycopene in the stroma and thus red colour develops in tomato fruit (Paliyath and Murr, 2008).

Lurie et al. (1996) reported that phytoene synthase1 (*PSY1*) gene which encodes fruit specific phytoene synthase was down-regulated during chilling. Bird et al. (1991) observed that down-regulation of *PSY1* resulted in yellow tomato devoid of lycopene. Not only *PSY1* gene, but also that gene expression of at least three other enzymes involved in carotenoid biosynthesis (carotenoid isomerase-*CRTISO*, geranylgeranyl diphosphate synthase 2-*GGPPS2* and 1-deoxy-D-xylulose-5-phosphate synthase-*DXS*) were down-regulated in chilled tomatoes (Rugkong et al., 2011). They further indicated that down-regulation of a MADS-box transcription factor necessary for fruit ripening, *LeMADS-RIN* (responsible for conferring nonripening phenotype of the *rin* mutant) was down-regulated

after 4 weeks chilling at 3 °C and this reduced expression contributed to the chilling-induced delayed ripening.

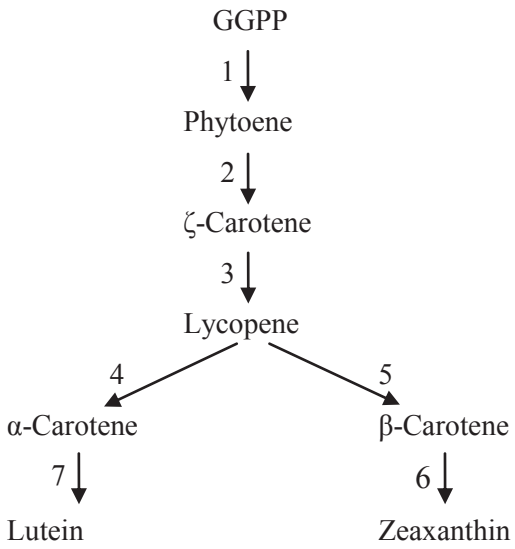

Figure 1.3 Summary of the biosynthetic pathway for carotenoids. Numbers indicate enzymes responsible for the conversion. 1. Phytoene synthase. 2. Phytoene desaturase. 3. ζ-Carotene desaturase. 4. β-Cyclase. 5. β- and *ε*-Cyclase. 6. β-Hydroxylase. 7. β- and *ε*-Hydroxylase (Source: Alexander and Grierson, 2002).

While reduction in expression of carotenoid synthesis genes during cool storage results in altered red colour development in chill-induced fruit, down-regulation of gene expression is possibly a function of chilling durations to which fruit are exposed. For example, Rugkong et al. (2011) found that tomatoes stored at 3 °C for 4 weeks showed reduced expression of *PSY1* and *CRTISO* compared to tomatoes at harvest. Expression of these genes increased after fruit were transferred to 20 °C following 1 week at 3 °C. When fruit were stored for 2 weeks, they showed increased expression of these genes during ripening at 20 °C but the expression was lower than in fruit stored for 1 week. Similarly, down-regulation in *GGPPS2* expression was observed with a longer chilling period. These results agree with the idea that for a short duration at low temperature tomato may be able to develop red colour whereas for longer duration tomato may fail to do so and instead show uneven blotchiness or yellow colouration.

A phenotype that shows uneven-blotchy red colouration during normal ripening has also been reported, although the cause of blotchy ripening is not clearly understood (Dr. J. Giovanonni, personal communication, *Ethylene 2012*). Blotchy areas are usually confined to the outer pericarp walls, but radial walls can also be affected in extreme cases (Yahia and Brecht, 2012). Some pre-harvest factors include low light intensity, cool temperatures, high soil moisture, high nitrogen, and low potassium or combinations of these factors, are thought to contribute to blotchy ripening (Yahia and Brecht, 2012). Blotchy areas of fruit walls contain less organic acids, dry matter, soluble solids, and starch sugar (Adams et al., 1978), indicting some kind of disturbed metabolism. Molecular work suggested that over-expression of a fruit ripening booster (*FRB*) gene, an auxin response factor, caused accelerated and patchy ripening (Breitel, 2012). Down-regulation of *DR12* (developmentally regulated clones), another auxin-response-factor homolog, in the tomato resulted in a pleiotropic phenotype including dark green and blotchy ripening fruit (Jones et al., 2002). While over-expression of some genes may cause a blotchy phenotype, it remains unclear whether blotchy uneven ripening induced by CI is related to expression of those genes. Blotchy ripening caused by *FRB* appeared to have quite sharp boundaries between green and red tissues (Breitel, 2012), unlike CI where the colour was more diffused with patches ranging from yellow to red on fruit surface.

Overall, when tomato is exposed to low temperature for a certain period of time, fruit exhibits abnormal colour development. Various factors may play a role in inducing blotchy colour development including modification of organelles such as chloroplasts or the inhibition of enzymes activities in biosynthetic pathway to lycopene or degradation of chlorophyll, down-regulation of genes encoding those enzymes or over-expression of some genes related to blotchiness.

1.3.2. Abnormal texture

1.3.2.1. Tomato textural properties and measurement techniques

Tomato is a fleshy fruit (a berry) surrounded by a pericarp walls which includes the inner wall, columella, radial wall, septa and the outer wall (Figure 1.4). Tomato “skin” or epidermis is composed of a thin layer of heavily cutinized epidermal cells. Vascular bundles distributed throughout the peripheral pericarp and columella run from the stem end to the blossom end. In the early stages of fruit development, locules are firm and compact.

With fruit maturation the parenchymatous tissue becomes gelatinous; cell walls become thin and eventually rupture to produce the locular gel. Locular cavities of the ripe tomato fruit are filled with a jelly-like material and seeds (Brecht, 1987).

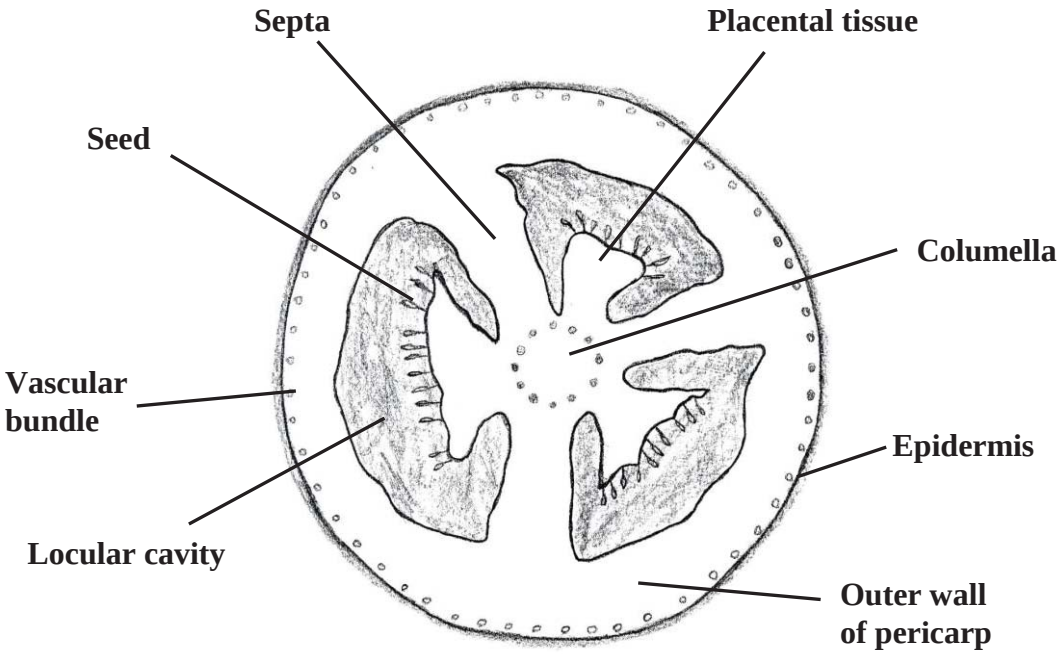

Figure 1.4 Transverse section of a three locule tomato fruit

Factors affecting textural properties of tomatoes include:

1. cell wall composition and activity of softening-related enzymes
2. turgor pressure, as dictated by water status, and cell membrane integrity
3. cell shape and size; larger cells tend to have greater strain in their walls and are thus somewhat more susceptible to cracking or fracture than smaller cells
4. amount and distribution of intercellular spaces
5. proportion and arrangement of specialised tissues such as vascular, epidermal, and locular tissues

Tomato softening can be a consequence of loss of turgor, loss of cell wall rigidity by degradation of polymer constituents of the cell wall or reduction of cell to cell adhesion caused by solubilisation and depolymerisation of the pectin rich middle lamella (Huber, 1983; Shackel et al., 1991). Various methods are used to evaluate changes in textural properties including puncture, compression, extrusion, shear, and others (Bourne, 1994)

and it is important to know which measurement techniques determine what characteristic of textural properties. Some of these methods apply a large deforming force (e.g. via puncture) and are therefore destructive. On the other hand, some tests that apply a small amount of deformation or force have usually been considered as non-destructive (e.g. compression firmness). The puncture test is the most frequently method used for textural evaluation, by measuring the force required to push a probe into a fruit to a specific depth; this causes irreversible damage or failure (Jackman and Stanley, 1995). Therefore, when a probe is punctured through the flesh of a pericarp, it possibly measures cell wall rigidity, cell packing and cell-cell adhesion. As tomato texture depends not only on flesh firmness, but is also influenced by skin toughness and the ratio of pericarp/locular material (Grierson and Kader, 1986), it is important to know whether puncture was performed from outside the epidermis and inside a tomato pericarp without locular gel. Flat plate compression usually squeezes (compress) the fruit and measures fruit turgor and overall fruit firmness. Although flat plate compression firmness is widely used to measure tomato firmness, Jackman and Stanley (1995) noted that compression measurement of tomato fruit may not be very sensitive to the tissue properties as such. Acoustic stiffness sensor or sonic transmission, another commonly used method, is based on resonance theory (any body that possesses both mass and elasticity is capable of vibrating). Depend on the specific physicomechanical properties of the fruit, free vibration may be exhibited at one or more frequencies and fruit firmness is mainly correlated with the two lowest frequencies. Overall elastic behaviour or stiffness factors are commonly used as indices of textural quality (Jackman and Stanley, 1995). Use of microscope to study fruit texture, structure or arrangement of tissue type has also been reported (Barrett et al., 1998).

1.3.2.2. Changes in textural properties during chilling and ripening

Low temperature storage alters tomato textural properties resulting in unusual changes in firmness. Different research groups observed variation in chilling effect on firmness changes including increased softening (Efiuvwevwere and Thorne, 1988), inhibited softening (Rugkong et al., 2011) or both excessive softening and persistent firmness following exposure to low temperatures (Hobson, 1987). Inconsistent reports of changes in firmness of tomatoes stored at chilling temperatures could be due to differences in the definition of firmness, in methods used to measure firmness, and in the degree to which fruit had been chilled (Jackman et al., 1990). Chilling related softening could be attributed

to formation of pectate salts (Buescher, 1974), associated changes in ion leakage (Autio and Bramlage, 1986) and loss of tissue integrity (Moline, 1976) and/or altered cell wall metabolism (Rugkong et al., 2010) which could be influenced by exposure time and the extent of chilling temperature (Efiuvwevwere and Thorne, 1988).

During normal ripening, softening in tomato is characterised by a reduction in cellular turgor pressure (Shackel et al., 1991) and catabolism of different cell wall components (Brummell and Harpster, 2001). Cell wall disassembly is a complex process involving both enzymatic and non-enzymatic mechanisms. Studies show that many classes of cell wall-modifying enzymes coordinate this cell wall modification in a synergistic manner, including polygalacturonase (PG), pectin methylesterase (PME), β-galactosidase (β-gal), endo-1,4-β-glucanase (EGase), xyloglucan endotransglucosylase/hydrolase (XTH) and expansin (exp) (Brummell and Harpster, 2001). PG, which is the most abundant pectin-degrading enzyme, catalyzes hydrolytic cleavage of pectin polymer homogalacturonan (Brummell and Harpster, 2001); while PME catalyzes pectin demethylation which in turn makes the pectin more susceptible to the action of PG (Pelloux et al., 2007). β-galactosidase plays a role in hydrolysis of galactan side chains of pectic polysaccharides, while expansin (Exp) is thought to bring about loosening of xyloglucan-cellulose network and alters the access of other cell wall enzymes to their substrates (Brummell et al., 1999). Xyloglucan endotransglucosylase-hydrolase is thought to contribute to xyloglucan depolymerisation (Saladie et al., 2007). Most of these enzymes exist in multigene families with a subset of one or more gene family members regulating this cell wall modification process.

Pectin is the most abundant class of macromolecules within the cell wall matrix and in the middle lamella between primary cell walls. During fruit softening pectin typically undergoes solubilisation and depolymerisation that are thought to contribute to cell wall dissociation through loosening and disintegration (Brummell and Harpester, 2001). Textural changes occurring in chill-injured tomatoes differ from those of normally ripening fruit specifically by altering pectin dissolution (Almeida and Huber, 2008). A reduction of pectin solubilisation and depolymerisation can be attributed to a higher CI incidence in tomato (Rugkong et al., 2010). However, pectin depolymerisation is not a ubiquitous requirement for pectin solubilisation (Brummell, 2006). Reduction of pectin solubilisation and absence of pectin depolymerisation may contribute to the abnormal texture of chill-

injured tomato fruit (Jackman et al., 1992; Almeida and Huber, 2008). Reduced pectin solubilisation and polymerisation in chilled fruit has been observed in peaches (Brummell et al., 2004), nectarines (Dawson et al., 1995), and plums (Manganaris et al., 2008). More importantly, degree of chilling-induced reduction of pectin solubilisation and polymerisation could differ depending on the extent of chilling damage (Rugkong et al., 2010).

For many years PG was thought to be the primary enzyme responsible for tomato fruit softening (Prusky, 1996). PGs catalyze the hydrolytic cleavage of galacturonide linkages (Giovannoni et al., 1989) and are largely related to pectin depolymerisation and solubilisation (Villarreal et al., 2008). However, their role in cell wall depolymerisation and solubilisation is a subject of debate (Brummell and Labavitch, 1997; Almeida and Huber, 2008). Reduced PG activity has been reported in chilled tomato (Marangoni et al., 1995) and other fruit, such as mango (Kesta et al., 1999). Molecular studies revealed that with increasing exposure to chilling temperatures, chilled tomato showed a higher reduction in transcript abundance, total activity, and protein accumulation encoding PG than non-chilled fruit (Rugkong et al., 2010) and inhibited softening after prolonged storage (Watkins et al., 1990). However, work with transgenic plants had introduced doubt as to the exact association between cell wall degradation caused by PG and tomato fruit softening (Giovannoni et al., 1989). Suppression of PG activity resulted in fruit with altered pectin metabolism but similar softening to controls in transgenic tomatoes (Smith et al., 1988). Jackman et al. (1992) speculated that softening of chilled fruit after transfer to a higher temperature was induced by non-extractable PG. Almeida and Huber (2008) suggested that PG was not major determinant of softening of chilled fruit as there was no PG accumulation in chilled tomato. While increasing PG activity in transgenic *rin* (ripening inhibitor) fruit caused increased pectin solubilisation (Giovannoni et al., 1989), and transgenic tomato suppressed in PG activity showed reduced water-soluble pectin (Carrington et al., 1993), other experiments with PG-antisense fruit have shown no correlation between pectin solubilisation and PG (Brummell and Labavitch, 1997). Huber (1983) found that some fruit can soften in the absence of dramatic increase in PG activity and Lurie et al. (1992) suggested that the levels of PG mRNA did not correlate well with the measured firmness of tomato. Jackman et al. (1992) and Marangoni et al. (1995) reported that enhanced softening in tomatoes was not correlated with PG activity; however,

it was associated with higher PME activity especially upon removal of tomatoes from cool storage.

Although PMEs play a minor role in fruit softening during ripening, their effect on cell wall tissue integrity and rigidity is important (Brummell and Harpster, 2001). Low PME expression in colourless non-ripening (*Cnr*) tomato mutants is thought to be responsible for maintaining a strong cell wall, indicating its role in maintaining fruit cell wall integrity (Eriksson et al., 2004). PME activity in chill-injured fruit is also a matter of debate. Both reduced PME activity (Buescher and Furmanski, 1978) and increased PME activity (Brummell et al., 2004) were reported for chilled stone fruit whereas in tomato, Rugkong et al. (2010) observed that PME activity was not affected by cool storage. Artés et al. (1996) attributed chill-induced woolliness in peaches and nectarines to low PG and continuous PME activity. It was suggested that demethylation of pectins by PME and inhibition of PG (particularly endo-PG) activity during low temperature storage resulted in an increase in the level of high molecular weight low methoxyl pectins that hold water in gel and induce woolliness (Fernández-Trujillo et al., 1998; Zhou et al., 2000).

Cell wall metabolism of tomato fruit is affected by exposure to CI-inducing temperatures, in some ways that are similar to those associated with mealiness development in stone fruit (Rugkong et al., 2010). Mealiness has been reported as a CI symptom in tomato (Jackman et al., 1992), and has been described as a dry, grainy, coarse look on the cut surface (Ahrens and Huber, 1988). Ultrastructural evidence shows an extensive dissolution of the pectin-rich middle lamella in chill-injured tomato (Marangoni et al., 1989) and fruit soften at an accelerated rate relative to that of non-chilled fruit (Marangoni et al., 1995; van Linden et al., 2008). Chill-injured tomatoes then develop mealiness where water was translocated to the modified wall resulting in a dry mouth feel of chilling injured fruit (Jackman et al., 1992).

Inhibition of fruit softening has also been reported in chilled tomato (Rugkong et al., 2010). Reduced expression of genes encoding *PG, PE1* (pectin esterase), and *LeExp1* contributes to reduced softening in chilled tomato (Rugkong et al., 2011). Reduced expression of gene encoding expansin or PG was also observed in cold-stored bananas (Wang et al., 2006) or avocados (Dopico et al., 1993). However, it is unclear whether inhibition of softening was simply a result of delayed ripening due to chilling.

Results reported here suggest that exposure of CI-inducing temperature alters tomato texture although inconsistent results have been found. Various factors may contribute to these discrepancies –

1. First, differences in extent of CI damage contribute to the difference in degree of reduction in pectin solubilisation and depolymerisation and may explain, at least in part, different textures found in chill-injured tomato. Duration of chilling exposure could further explain the discrepancies experienced in the different studies.
2. Second, during chilling gene expression of cell wall disassembly associated enzymes could be down-regulated (*PG, PE1, LeExp1*), up-regulated (xyloglucan endotransglucosylase-hydrolase 5, *LeCBF1* cold-response gene - C-repeat/dehydration-responsive element binding factor) or without any effect (*TBG4* and *Cel1*) (Zhao et al., 2009a; Rugkong et al., 2010). These may potentially have differential effects of chilling on different broad classes of matrix glycans and thus contribute to inconsistent results in chilling-induced tomato softening.
3. Third, fruit maturity may play a role in having different effects on texture during chilling. For example, Roy et al. (1992) showed that methylesterification was controlled both in time (during ripening) and space (location in the wall) in tomato. In green fruit, pectin in most of the wall had a high degree of methylesterification, except for the middle lamella. As ripening progressed, regions of low methylesterification spread inwards from the middle lamella, until by the red ripe stage represents specific pectin with low methylesterification were spread throughout the wall (Roy et al., 1992; Steele et al., 1997).
4. Fourth, the way fruit firmness is measured, whether measured softening is a result of loss of turgor or loss of tissue integrity, and the degree of CI severity (mild or severe), are all important factors that can contribute to the inconsistent results reported in determining chilling-induced tomato softening.

In short, severity of chilling damage, duration of low temperature exposure, fruit maturity and activity of many enzymes and associated gene expressions in a particular chilling temperature have to be considered before the mechanism of softening in chilling-induced tomato can be elucidated. More importantly, since both ripening and chilling induce fruit softening; it is important to differentiate chilling-induced softening from softening

associated with ripening in determining abnormal texture that is usually reported as a result of low temperature storage.

1.3.3. Increased susceptibility to decay

Increased susceptibility to decay is another CI symptom in tomato. *Alternaria*, *Phytophthora, Botrytis, Geotrichum* are some of the major postharvest fungi and *Erwinia, Pseudomonas* and *Xanthomonas* are common bacterial pathogens in tomato (Fallik et al., 1993). During normal ripening susceptibility to decay increases as tomatoes ripen (Fallik et al., 1993; Prusky, 1996). However, mature-green tomatoes stored at a temperature below 12 °C for extensive periods are prone to disease susceptibility (Artés and Escriche, 1994). Although it is possible that mechanisms of ripening associated-decay development differ from chilling-induced decay development, increased decay susceptibility related to ripening and chilling could be confounded during low temperature storage.

Among the different fungi, *Alternaria* was identified as the most frequently isolated fungi in chill-injured tomatoes (Efiuvwevwere and Thome, 1988). *Alternaria* is a weak pathogen that usually develops on the stem scar (Hall, 1965) as resistance to pathogens is easily circumvented due to unavoidable wounding related to harvest. In the case of *Geotrichum*, entry is usually by stem scar, wound or weak epidermal tissue or in case of *Fusarium* - infection is generally at a wound site (Baldwin et al., 2011). Additionally chilling-induced pitted tissue is often ideal entry point for microorganisms. In many cases fungus enter through damage of skin caused by mechanical damage (Barkai-Golan, 2001).

Many factors play a vital role in inducing increased susceptibility or a resistance against pathogens. When a fruit is exposed to pathogen attack, tissues produce and deposit lignin and suberin in their wall as a protective barrier to counterattack that stress (Eckert, 1978). Temperature and humidity are important factors for suberisation during postharvest storage. Maintaining fruit at low temperatures below the chilling threshold or for too long could prevent the formation of suberin by cells surrounding the wound and ultimately facilitate infection of an intact host (Barkai-Golan, 2001).

Links between ethylene and decay incidence have been proposed. However, ethylene has an ambiguous effect on decay development and the mechanism of ethylene involvement in

enhancing tissue susceptibility to pathogens is a matter of debate (Porat et al., 1999). Ethylene application stimulated *Alternaria* and *Botrytis* in tomatoes (Segall et al., 1974; Barkai-Golan and Lavy-Meir, 1989) or *Botrytis* in strawberry (El-Kazzaz et al., 1983). Even concentrations of ethylene lower than those produced during ripening of climacteric fruit are capable of stimulating pathogen hyphae germination and elongation (Flaishman and Kolattukundy, 1994). It is possible that ethylene promotes fruit ripening and ripening is characterised by an increase in susceptibility to necrotrophic pathogens (Prusky, 1996; Giovannoni, 2001). On the other hand, removing ethylene with potassium permanganate reduces susceptibility to *Botrytis* (Wills and Kim, 1995). Su and Gubler (2012) demonstrated that application of 1-MCP reduced postharvest decay in mature-green tomatoes stored at 18 °C.

However, some researchers reported that ethylene does not affect disease development, and may even enhance host resistance to pathogen attack by activating plant defence related processes including production of phytoalexins (Fan et al., 2000) or pathogenesis related proteins (Ding et al., 2002). Geeson et al. (1986) found that tomatoes stored under controlled atmospheres showed increased susceptibility to *Botrytis* with a decrease in ethylene supply and suggested that 1-3 $\mu L.L^{-1}$ of ethylene was required for decay control. Similarly, resistance to decay by ethylene was reported for sweet potatoes infected by *Ceratocystis fimbriata* (Stahmann et al., 1966) or tangerines inoculated with *C. gloeosporioides* (Brown and Barmore, 1977). Diáz et al. (2002) suggested that ethylene perception is required for increased resistance of tomato leaves to *Botrytis*, as inhibition of ethylene response by 1-MCP resulted in increased decay susceptibility. 1-MCP also resulted in increased decay susceptibility in mature-green tomatoes stored at 3 °C (Jian and Ze-Sheng, 2011), consistent with ethylene involvement in reducing decay during cool storage.

Results reported here indicate that ethylene may have a positive effect in reducing decay in many crops. In some cases, however, it may have a detrimental effect and thus exposure to ethylene should be avoided. Inconsistent results obtained of this relationship between ethylene and decay susceptibility during storage may be because of different fruit tissues vary in their response to ethylene. More importantly, it is necessary to determine whether perceived decay is chilling-induced or ripening associated. It is possible that the positive effect of 1-MCP in reducing ripening-associated decay could be different from chilling-

induced decay reduction by 1-MCP. For example in tomato, since 1-MCP inhibits or delays fruit maturity, its effect when applied to mature-green fruit may make fruit more sensitive to chilling than untreated fruit. Thus, it is possible that ethylene is beneficial in reducing chilling-induced decay. On the other hand, decay susceptibility increases as fruit ripens, so by arresting the advancement of ripening, 1-MCP possibly reduces decay during normal ripening.

Chilling induces cell wall disassembly which may contribute to decay susceptibility in fruit by altering the structure or the accessibility of cell-wall substrates to pathogen cell wall degrading enzymes (Cantu et al., 2008). Many saprophytic and plant pathogenic organisms secrete cell wall degrading enzymes that specifically target components of the cell wall so that pathogens can penetrate the host and acquire nutrients from digested wall material and cellular contents (Collmer and Keen, 1986). Ripening impaired tomato mutants *Nr* or *nor* showed reduced ripening-associated softening and decreased susceptibility to pathogens (Lavy-Meir et al., 1989; Kramer et al., 1992). However, cell wall disassembly alone cannot explain pathogen susceptibility in tomato. Suppression of the ripening associated expansin gene, *LeExp1*, resulted in firmer fruit with prolonged shelf life, but did not reduce susceptibility to *Botrytis* and *Alternaria* in tomato. Cantu et al. (2008) suggested that simultaneous suppression of both *LePG* and *LeExp1* expression is an important determinant of the ripening-associated increase in decay susceptibility.

Overall, there are various mechanisms that exist if a pathogen attacks any host. A pathogen, when it finds suitable conditions for penetration and establishment within host tissues, will cause a disease only if it succeeds in circumventing the protective barrier of the host (Barkai-Golan, 2001). Factors that help pathogens to defeat the host barrier include ability to produce cuticle- and cell wall-degrading enzymes or produce toxic compounds. On the other hand, defence mechanisms of the host also depend on the ability of the intact cuticle to provide a barrier to fungal penetration including the ability to produce antimicrobial activities and phytoalexins or the capability of wound healing and strengthening of cell wall by formation of glycoproteins, callose, lignin and other phenolic polymers. Nevertheless, if fruit tissue have already become “weak” due to avoidable wounding related to harvest, postharvest abuses including poor handling practices and improper temperature management may make fruit tissue too vulnerable to avoid the pathogenic attack.

1.3.4. Loss of aroma and flavour

Uneven blotchy red colouration, complete failure to develop normal red colour, abnormal texture and increased decay susceptibility are all gross CI symptoms, whereas loss of aroma and flavour is a symptom of very subtle chilling damage but nonetheless important in terms of quality (Maul et al., 2000). It is usually accepted that ripe tomatoes purchased in supermarket lack the desirable "tomato-like" flavour compared to ripe tomatoes picked directly from the field (Buttery et al., 1987; Baldwin et al., 2008). Breeders have focussed predominantly on traits related to higher yield, visual characteristics, disease resistance, as well as larger and firmer fruit (Maul et al., 2000; Tieman et al., 2006). Sensory quality such as aroma and flavour has been neglected over time. A complex nature and large number of genes contributing to the flavour trait are other reasons for not having received emphasis by researchers (Mathieu et al., 2009). However, the development of molecular tools offers new opportunities to introduce genes that enhance aroma without compromising other important traits (Galili et al., 2002).

Sugars, acids, and their interactions are important to sweetness, sourness and overall flavour intensity in tomatoes (Stevens et al., 1977; Baldwin et al., 2008). The pleasant sweet-sour taste of tomatoes is mainly because of their sugar and organic acid contents. Storage temperature plays a significant role in maintaining aroma and flavour of tomato (Kader, 1986). Low temperature storage alters the ratio of sugars and acids in tomato (Hall, 1968) and changes perceived flavour (Maul et al., 2000). Accumulation rate of citric acid and acetic acid increased as storage temperature was reduced but decreased at higher temperature (19 °C) (Thorne and Efiuvwevwere, 1988). Mature-green fruit stored at 2 °C for 14 - 21 d (Kader et al., 1978) or breaker fruit stored at 6 °C for 27 d (Gómez et al., 2009) had lower concentrations of both glucose and fructose than non-chilled fruit. Therefore, lower flavour intensity of refrigerated tomatoes may result from higher acidity and/or lower sugar. Since mature-green tomatoes have a lower sugar: acid ratio than those harvested at ripe stage (Moneruzzaman et al., 2008), it is not immediately obvious if alteration in the sugar: acid ratio following low temperature exposure reported by these researchers is a direct effect of chilling or simply an indirect effect of delayed ripening.

Tomato flavour results from a combination of taste components, aroma compounds and a complex interaction between them (Petro-Turza, 1987). The five tastes - sweet, sour, salty, bitter, and umami, are perceived by certain regions of the tongue, while volatiles are

perceived by the olfactory nerve endings of the nose (Acree, 1993; Marcus, 2009). With more than 400 volatile compounds identified in tomato, only 30 are present in concentrations over one ppb (Petro-Turza, 1987). Buttery et al. (1971) determined odour thresholds (the level at which a compound can be detected by smell) for these 30 compounds. Of these, only 16 have 'positive' log odour units and are likely to contribute to characteristic "tomato flavour" (Baldwin et al., 2000). Hexanal, *cis*-3-hexenal, *trans*-2-hexenal, 1-hexanal, *cis*-3-hexan-1-ol, hexanol, 2-isobutylthiazole, 6-methyl-5-hepten-2-one, and methyl salicylate are considered the most important flavour-contributing impact factors (Buttery et al., 1987; Petro-Turza, 1987). These flavour compounds are believed to be synthesised from a diverse set of precursors, including lipid derivatives (hexanal, hexanol, *cis*-3-hexenal, *trans*-2-hexenal, 1-hexanal, *cis*-3-hexanol, 2-isobutylthiazole), amino acids (3-methylbutanol and 3-methylbutanal), lignins (guiacol), and carotenoids (β-ionone, geranylacetone) through different biochemical pathways (Sanz et al., 1997; Tieman et al., 2006).

Evidence of the adverse effects of low temperature storage on tomato flavour has been documented elsewhere (Kader et al., 1978; McDonald et al., 1996; Maul et al., 2000; Boukobza and Taylor, 2002; Díaz de León-Sánchez et al., 2009; Bai et al., 2011). Loss of flavour occurs before even other symptoms of chilling become apparent (Kader et al., 1978). The modification of aroma compounds in cool-stored tomatoes could be related to alterations in the availability of the precursor contents or reduction of enzyme activity in the biosynthesis of aroma compounds (Boukobza and Taylor, 2002).

Low temperature storage often alters or inhibits red colour development of mature-green tomatoes (Hobson, 1987) and subsequently lowers flavour intensity particularly by reducing carotenoid-derived compounds concentrations (Baldwin et al., 2000). Tomatoes with a 'high lycopene' content are believed to have a high aroma and flavour (Kader, 1986), who reported that high beta-carotene cultivars (e.g. 'Caro Red') and high delta-carotene cultivars (e.g. 'Gold Jubilee') had a distinct volatile composition and flavour. When tomato fruit were cultured *in vitro* and treated with 2-(4-chlorophenylthio)triethylamine (lycopene β-cyclase inhibitor, which usually enhances lycopene production and chromoplast differentiation in many higher plants), lycopene content increased and so as did carotenoid-derived aroma compounds such as 6-methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol (Ishida et al., 1998). On the other hand, fruit

from plants containing antisense PSY had a lower level of carotenoid-derived volatiles (6-methyl-5-hepten-2-one, geranylacetone and *β*-ionone) compared with wild type tomatoes (Baldwin et al., 2000). Since chilling temperature affects colour development in tomato and volatile composition of tomato is closely related to fruit colour (Stevens, 1970; Gao et al., 2008), it is important to determine whether chilling-induced alteration in flavour is independent of delayed ripening.

Ethylene plays an important role in enhancing biosynthesis of aroma compounds in ripening fruit (Alexander and Grierson, 2002). Since the biosynthetic pathway of ethylene production is related to biotic and abiotic stresses (Watkins and Ekman, 2004), it is likely that altering ethylene production during cool storage results in changes in aroma development. Ethylene evolution results in enhancing biosynthesis of aroma compounds such as hexanal, *cis*-3-hexenal, *cis*-3-hexenol, and *trans*-2-hexenal during ripening (Baldwin et al., 1991). Generally ethylene affects essential enzymes involved in biosynthetic pathways that influence the concentration of volatiles, including lipoxygenase (LOX), alcohol dehydrogenase (ADH) and alcohol acyltransferase (AAT) (Zhu et al., 2005; Díaz de León-Sánchez et al., 2009). Lower concentrations of aroma compounds in chilled tomatoes could be due to lower enzyme activity and reduced expression of genes encoding those enzymes (Bai et al., 2011).

A relationship between ethylene production, colour development and enhanced biosynthesis of aroma compounds during ripening has been suggested (Alexander and Grierson, 2002; Zhu et al., 2005). In tomato, inhibition of ethylene production by suppression of ACS hinders normal ripening and reduces aroma volatiles significantly (Oeller et al., 1991) in particular those that are carotenoid-derived (Gao et al., 2007). Transgenic melon fruit with antisense ACO treated with 1-MCP demonstrated a 50% reduction in ATT activity and ultimate aroma development (Flores et al., 2002). Application of AVG (ReTain^{TM} - an ethylene synthesis inhibitor) or diazocyclopentadiene (an ethylene action inhibitor) have shown that the biosynthesis of ester volatiles by ripening apples requires a high rate of ethylene production (Fan et al., 1998). However, inhibiting of ethylene action by 1-MCP did not affect tomato flavour dramatically (Mir et al., 2004), especially when pink maturity tomatoes were treated with 1-MCP (Cliff et al., 2009). Similarly, Baldwin et al. (2011) did not find any detrimental effect of 1-MCP on flavour of breaker tomatoes. It appears that in tomatoes which have reached a certain

maturity stage (i.e. breaker/pink), delaying ripening will be less deleterious and perhaps sensory qualities have already developed by that stage (Guillén et al., 2006).

Fatty acids are considered key precursors in formation of tomato aroma volatiles (Wang et al., 1996). Since chilling alters lipid saturation, possibly there is a connection between chilling-induced lipid composition changes and aroma development following low temperature stress. Many flavour compounds are derived from peroxidation of unsaturated fatty acids through the lipoxygenase/lyase enzyme pathway (Stone et al., 1975; Vick and Zimmerman, 1986). Cleavage of 13-hydroperoxide from linoleic (18:2) and linolenic (18:3) acids produces hexanal and *cis*-3-hexenal respectively. Both hexanal and hexenal can further be reduced by ADH to form hexanol and *cis*-3-hexenol (Vick and Zimmerman, 1986). *Cis*-3-hexenal can further be isomerised to *trans*-2-hexenal with or without enzyme activity (Riley et al., 1996).

Yilmaz et al. (2001) reported that enzyme activity itself was not a good predictor of the amount of volatiles produced and suggested that availability of precursors and composition may be more important in determining fruit flavour composition. For instance, Sirit et al. (2007) found that availability of appropriate unsaturated fatty acids is an important factor affecting fruit flavour and manipulation of these fatty acid metabolic pathways has been attempted by the expression of a yeast Δ-9 desaturase gene. Modifying expression of ω-3 desaturase in tomato results in an increase in the 18:3/18:2 ratio and altered aroma profile in addition to enhanced chilling tolerance (Dominguez et al., 2010). These findings, therefore, suggest that there is likely to be a direct connection between chilling induced fatty acid composition changes and aroma development in tomato.

Adverse effects on tomato flavour after low temperature storage are documented elsewhere. Low temperature usually lowers ethylene production, alters red colour development and delays ripening. Therefore, apparently it is unclear whether lower concentrations of some aroma compounds reported in cool-stored tomatoes are due to direct effect of low temperature stress or indirect effect of fruit's delayed ripening. It can be argued that flavour loss of mature-green tomatoes after storage could be because of, at least in part, failure of fruit to ripen. Many researchers recognised these issues and they used advanced maturity stage tomatoes since it is believed that organoleptic qualities have already developed by that stage. Therefore, for example, if lower concentrations of

carotenoid-derived volatiles are found in chilled tomatoes harvested at advanced maturity stages (pink or ripe stage) than in non-chilled fruit as reported by Maul et al. (2000) or Boukobza and Taylor (2002), it can be argued that this is a direct result of chilling. Nonetheless, aroma development is by no means complete when tomatoes are harvested at breaker or pink stage and low temperature storage can cause delayed ripening in tomatoes even harvested at advanced maturity stages (Gómez et al., 2009). Therefore, reduced concentrations of aroma compounds in those chilled tomatoes reported by scientists may not be simply a result of direct effect of chilling but could be just a delayed ripening.

1.3.5. Increase in ion leakage – indicator of membrane damage

An increased rate of solute leakage in tissues is often correlated with appearance of CI symptoms (Saltveit, 2002) and measurement of CI severity in many crops including tomato (Saltveit, 1990; Lafuente et al., 1991; Côté et al., 1993). The rate of increase in electrolyte leakage varies with season, crop, and also with cultivar (Kuo and Parkin, 1989; Saltveit, 2005). An enhancement in electrolyte leakage was reported in tomato ('Castlemart'; harvested in summer) pericarp discs chilled at 2.5 °C after 3 d (Saltveit, 2002). However, pericarp discs from the same cultivar harvested during winter exhibited an increase in electrolyte leakage only after 6-7 d at 2.5 °C (Saltveit, 2005).

Chilling does not immediately increase the rate of ion leakage from tomato pericarp disc rather it causes a progressive increase in permeability over a few days of chilling (Saltveit, 1989). In cucumber stored at 4 °C, irreversible damage due to chilling required 7 to 10 d as indicated by increases in tissue electrolyte leakage (Kuo and Parkin, 1989). Since several days of chilling are required for leakage rates to become significantly greater than for the non-chilled control (Saltveit, 2002), it suggests that the phenomenon may not be a direct result of an abrupt temperature-induced 'phase-transition'. Because if phase changes are the direct cause of chilling induced leakage, changes in membrane permeability should be rapid and leakage should be detectable within a few minutes of chilling. Nonetheless, it may be possible that underlying damage in cellular membrane may occur due to initial abrupt lipid phase transition after an exposure to low temperature, but the significant increase in ion leakage may take longer to display. Interestingly, in some species (*Episcia*, *Achimines*, and *Gloxinia*) injury can occur after only a few hours at 1 to 5 °C (Seible, 1939, as cited in Lyons, 1973).

Fruit maturity has a large influence on membrane permeability and solute leakage. Red ripe tomatoes have a higher electrolyte leakage than mature-green tomatoes (King and Ludford, 1983) and a ripening related increase in electrolyte leakage is well documented in many other crops (Lewis and Martin, 1969; Murata, 1990). Consequently increase in electrolyte leakage associated with ripening can be confounded with the residual change in electrolyte leakage that reflects chilling damage. It is necessary to differentiate the increase in electrolyte leakage associated with ripening from that which indicates chilling-induced damage. More importantly, it is usually believed that chilling sensitivity reduces as fruit ripen (Autio and Bramlage, 1986). Therefore, with advancement of ripening, fruit is expected to decrease chilling-induced ion leakage. It is possible that there is an extensive cross-talk between chilling-related injury and ripening-associated changes in fruit physiology.

There are also some inconsistencies found in terms of electrolyte leakage measurement as an indicator of CI. Bergevin et al. (1993) found a decrease in electrolyte leakage after tomato fruit chilled at 1 °C for 14 and 18 d were returned to 20 °C, when CI symptoms usually appears. Côté et al. (1993) noted that electrolyte leakage decreased drastically when a chilling sensitive tomato variety was returned to 20 °C after 20 or 27 d at 3 °C.

Inconsistent results on chilling-induced ion leakage reported by many scientists could arise because not all studies follow some conditions necessary to accurately calculate rate of ion leakage. For example, Saltveit (2002) reported that three conditions must be followed to ensure accurate representation of membrane permeability – i) tissue should be submersed in an aqueous isotonic solution ii) rate of ion leakage must be linear during the sampling period and iii) if the tissue is freshly cut, it should be washed and an isotonic solution should be used to reduce the additional stress.

In brief, an increased rate of ion leakage is often considered an indicator of chilling-induced cellular membrane damage although lack of consistent results has been observed. Fruit maturity, cultivar, chilling exposure time-temperature interaction or, more importantly, measurement techniques can all contribute to these discrepancies. Despite such inconsistencies, an increased rate of electrolyte leakage has been used as an indicator of physical damage of cell membranes in fruit, including tomato (Saltveit, 2005; Antunes and Sfakiotakis, 2008; Zhao et al., 2009b; Dea et al., 2010).

1.4. Alleviation of chilling injury

It is well documented that chilling injury limits storage and marketability of many horticultural crops (Wang, 1990). Therefore, the ultimate goal of chilling injury research is to find effective techniques to alleviate this disorder. If tolerance of these sensitive tissues can be increased or development of CI symptoms can be delayed, then it would be possible to extend storage life, marketability and importantly allow export of fruit and vegetables by sea instead of more expensive air freight (Wang, 1993). Several techniques have been proposed to alleviate CI including high or low temperature conditioning, intermittent warming, controlled atmosphere, and applications of growth regulators or other chemicals (Wang, 1993). Some of them employed manipulation of storage temperatures and atmospheres while others involved direct treatment to the commodities with growth regulators and chemicals (Lurie, 2008; Sevillano et al., 2009). Many of these techniques are cumbersome to employ in commercial situation. With great diversity of horticultural crops, it is difficult to generalise the mechanism of CI induction and its alleviation by different techniques. Therefore, it has been found that some of the techniques are more effective on certain commodities than others, and the optimum conditions vary with different crops and even different genotypes in the same species (Wang, 1993).

1.4.1. Temperature conditioning

Temperature conditioning, (i.e. step-down cooling) for better acclimatisation to low temperatures, is a technique adopted as a commercial practice in selected crops to reduce CI (Hatton, 1990). In low temperature conditioning, commodities are generally exposed to temperatures slightly above the critical chilling range which leads to increasing tolerance to chilling exposure and delay in the onset of injury symptoms (Woolf et al., 2003). Low temperature conditioning induces an adaptive response to chilling stress through modification of various physiological and biochemical changes in fruit tissues (Wang, 1993). These modifications include reducing the loss of membrane phospholipids, increasing sugar, starch and proline content, and maintaining high concentrations of polyamines, squalene and long-chain aldehydes and increasing the ratio of unsaturated to saturated fatty acids (Wang, 1993). All of these modifications contribute to an increase in chilling tolerance.

Stepwise reduction of temperatures is effective in reducing CI in tomatoes (Gálvez et al., 2010). For instance, tomatoes can be acclimated to low temperature storage by successive exposure for 4 d to 12 °C followed by 4 d at 8 °C and 7 d at 5 °C (Marangoni et al., 1990). Similarly, positive results of low temperature conditioning in alleviating CI have been reported in grapefruit (Hatton and Cubbedge, 1982), papaya (Chen and Paull, 1986), sweet pepper and summer squash (Hardenburg et al., 1986), lemon (Houck et al., 1990), avocado (Woolf et al., 2003), and loquat (Cai et al., 2006b).

1.4.2. Heat treatments

Postharvest treatments with high temperatures of varying duration have been primarily used for insect disinfestations (quarantine), avoiding fungal rot and maintaining fruit quality (Lurie, 1998). However, high temperature treatments prior to cool storage have also been proposed to reduce CI in a number of crops (Klein and Lurie, 1992; Lurie, 2008; Luengwilai et al., 2012).

It has been hypothesised that exposure to one type of stress may induce some factors which can protect against another type of stress (Lurie and Klein, 1991). Exposure of plant tissues to high temperature stress at about 10-20 °C above the normal growing temperature stimulated synthesis of polypeptides known as heat shock proteins (HSPs) (Lafuente et al., 1991). These HSPs, ranging from 15 to 40 kDa, may function as molecular chaperones (Saltveit, 2002). While low temperature can alter the solubility and folding properties of many proteins, HSPs assist to protect against stresses by controlling the proper folding and conformation of both structural and enzymatic proteins (Vierling, 1991). Therefore, HSPs bind to unfolded or denatured proteins and prevent cell damage, reduce chromatin condensation and DNA breakdown, and suppress oxidative activity (Wang et al., 2001).

Different methods have been employed for application of heat treatments such as hot air (vapour heat and forced air), hot water dipping (HWD), and hot water rinsing and brushing (HWRB). Heating tomatoes in air for 48 h at 38 °C before placing them at 2 °C reduced CI and extended shelf life in tomatoes (Lurie et al., 1992; Sabehat et al., 1996). A short-term HWD (42 °C for 1 h) or long-term hot air treatment (2 d exposure to 38 °C) allowed mature-green ‘Sunbeam’ tomatoes to ripen normally without CI after 2 weeks at 2 °C (McDonald et al., 1996). Similarly, pre-storage heat stress with HWD (50 °C for up to 1

min) and HWRB (a short-term treatment of pink tomato at 52 °C for 15 sec) is an effective means to reduce tomato ('189') CI (Ilic and Fallik, 2005). Chilling injury in avocados can be prevented by using a short hot air treatment at 38 °C (up to 10 h), or by 30 min in HWD at a temperature range 39-42 °C (Florissen et al., 1996; Hofman et al., 2002). Superficial scald of apple can be reduced by pre-storage hot air treatments at 46 °C for 12 h (Klein and Lurie, 1990) or a HWD at 48 °C for 3 min (Jemric et al., 2006). Similarly, hot air or hot water treatment prior to cold storage have been suggested to reduce CI in mango (Kesta et al., 2000), persimmon (Woolf et al., 1997), pepper (González-Aguilar et al., 2000), pomegranate (Rahemi and Mirdehgan, 2004), banana (Promyou et al., 2008), and citrus (Holland et al., 2005; Rivera et al., 2007).

However, these heat treatments sometimes have negative influences and could exacerbate another disorder e.g. bitter pit in apple (Neven et al., 2000). High temperature sometimes delayed ripening of tomatoes even after the temperature stress was removed (Cheng et al., 1988). In addition, high temperature stress can diminish the quality of tomato fruit by causing tissue destruction and physiological dysfunction (Inaba and Crandall, 1988). For instance, heating 'Rhapsody' tomatoes in air at 38 °C for 24 h before placing them at 4 °C for 4 weeks caused severe heat-injury and fruit had lower quantities of lycopene and greater loss in antioxidants compared to fruit heated at 34 °C for 24 h (Soto-Zamora et al., 2005). McDonald et al. (1999) found that 'Sunbeam' tomatoes pre-treated with hot water treatments at 48 °C for 1 h showed higher electrolyte leakage and reduced flavour than non-treated tomatoes.

Overall, these results suggest that different heat treatments successfully reduce CI in many horticultural crops, although these treatments may have some detrimental effect in some occasions. Reduction of CI and changes in quality attributes of crops in response to heat treatments varies with cultivar, heating temperature and duration of exposure and the method of employing heat (Whitaker, 1994; Lurie, 2008). In addition, ripening state and growing conditions probably contribute to differences in fruit response to heat treatment (Soto-Zamora et al., 2005). It is important to test each cultivar at different heating regimes and not simply use what has been reported for other cultivars (Lurie, 2008). Since temperature gradient during warming extends from the surface to the centre of the fruit, fruit shape and size, which affect the uniformity of the treatment (Paull and Chen, 2000), must also be taken into account.

1.4.3. Intermittent warming

Intermittent warming is the interruption of low temperature storage with one or more short periods of warm temperature for various periods of time (Schirra and Cohen, 1999; Porat, 2004). IW has been shown to be beneficial in reducing CI and improving keeping quality of several horticultural crops during postharvest storage (Saltveit and Morris, 1990). IW has achieved commercial success in some selected crops like lemon (Cohen, 1988) and lime (Kluge et al., 2003). Beneficial effects of IW in reducing CI in many crops and the mechanisms by which IW reduces CI will be discussed in detail later in section 1.5.

1.4.4. Controlled atmosphere and modified atmosphere storage

Controlled atmosphere (CA) or modifying the storage environment i.e. modified atmosphere (MA), particularly CO_2 and O_2 concentrations surrounding the commodities, has a positive effect in preventing CI or delaying onset of symptoms (Wang, 1993). MA storage may be different from CA storage in that MA imposes less stringent atmosphere controls, but they are very similar in basic principles and their effects on the product (Pranamornkith, 2009). CA uses 'active' techniques to control and maintain gas composition while in MA storage, desired gas composition is initially created by either passive or active techniques, but no 'active' maintenance technique is applied thereafter (Vigneault et al., 2004).

Morris et al. (1982) found that mature-green tomatoes could be stored up to 7 weeks at 12.8 °C under 4% O_2, 2% CO_2 and 5% CO and fruit had acceptable quality (colour and firmness) during post-storage period at 20 °C. Chilling-induced internal breakdown and reddening of nectarines were almost prevented when nectarines were kept in 10% O_2 + 10% CO_2 (Lurie, 1992). Likewise, addition of 5% CO_2 to a low O_2 atmosphere reduced CI symptoms in peaches and nectarines while maintaining storage quality (Anderson et al., 1969). Avocados pre-treated with atmospheres of 3% O_2 for 24 h at room temperature showed lower respiration, ethylene production, and electrolyte leakage with reduced CI symptoms than untreated fruit following storage at 0 °C for 3 weeks (Pesis et al., 1994). Similarly, increased CO_2 and lower O_2 concentrations reduced CI in zucchini squash, lemon, grapefruit, guava, and nectarine (Mencarelli, 1987, Bertolini et al., 1991; Meir et al., 1995; Singh and Pal, 2008).

Although most commodities respond favourably to a decrease in O_2 level and/or an increase in CO_2 concentration, controlled atmospheres may be detrimental or sometimes have no effect in alleviating CI in some crops (Wang, 1993). If the concentration of O_2 and CO_2 percentage goes beyond those tolerated by the commodity, it can induce physiological disorders such as brown stain on lettuce, internal browning and surface pitting of pome fruit, or blackheart of potato (Kader et al., 1989). CO_2 concentration above 3-5% is not tolerated by most tomato cultivars and would cause CO_2 injury while low O_2 ($\leq$ 1%) would cause off-flavours, objectionable odours, and internal browning (Parsons et al., 1970; Deltsidis et al., 2011).

MA storage using low density polyethylene film was beneficial in extending storage life and reducing CI symptoms in many chilling sensitive crops (Kader et al., 1989). Bananas stored at 10 °C responded favourably with reduced CI when fruit were held in 0.04 mm thick polyethylene bags (Scott and Gandanegara, 1974). Packaging of peppers in perforated films also resulted in a delay in CI appearance compared to non-packaged control fruit (Meir et al., 1995). A positive effect from packaging in reducing CI was also found in avocado (Scott and Chaplin, 1978), grapefruit (Purvis, 1985), cantaloupe melon (Lester and Bruton, 1986), and mango (Pesis et al., 2000).

1.4.5. Plant growth regulators

Plant growth regulators influence a wide range of biochemical and physiological processes in plant tissue and thus may render tissues susceptible or tolerant to low temperature (Wang, 1993). Plant hormone ethylene is known for its diverse effects on biotic and abiotic stresses (Lin et al., 2009). The role of ethylene in influencing CI was discussed previously in this review (section 1.2.1.2.2).

Another important growth regulator, abscisic acid (ABA), accumulates in response to a number of environmental stresses and invokes protection mechanisms against different stresses including low temperature. Application of ABA prevented CI in cucumber seedlings and cotton plants (Wang, 1993). ABA plays an important role in control of stomatal closure and therefore in transpiration rate. Thus ABA was suggested to have a beneficial effect on CI where low-temperature stress was characterised by water deficit e.g. maize seedlings stored at 4 °C (Janowiak et al., 2002). Application of exogenous ABA

caused a significant decrease in electrolyte leakage, one of the important CI indicators, in leaves of tomato plant (Kim et al., 2002). Lafuente et al. (1997) correlated increased resistance to CI with increased contents of ABA in the flavedo of 'Marsh' grapefruit. However, the authors reported that changes in ABA did not correlate with CI during maturation of highly CI-susceptible 'Fortune' mandarin. More importantly, an increased level of ABA induced CI in some citrus cultivars (Gosalbes et al., 2004); indicating a role of ABA in reducing CI is cultivar dependent.

Polyamines (PAs) such as the tetramine, spermine (Spm), the triamine spermidine (Spd), or the diamine putrescine (Put) appear to be ubiquitous in living cells (Smith, 1985). PAs have been associated with a variety of regulatory processes ranging from promotion of growth and cell division to inhibition of ethylene production and senescence (Ben-Arie et al., 1982). Besides PAs have been demonstrated to reduce CI and acted as antisenescent agents in plant defence system against different types of stresses (Smith, 1985). Exogenous application of PAs induced resistance to CI in zucchini and 'McIntosh' apple (Wang and Kramer, 1990). PAs have antioxidant properties and act as scavengers of reactive oxygen species (Drolet et al., 1986). Various postharvest treatments (temperature conditioning, modified atmosphere packaging or hot water treatment) caused an increase in PA concentration and reduced or delayed the development of CI in zucchini (Wang, 1994), pepper (González-Aguilar et al., 2000), and pomegranate (Mirdehghan et al., 2007).

Other plant regulatory compounds such as salicylic acid (SA) or jasmonic acid (JA) were found promising to reduce CI in many crops include maize (Janda et al., 1999); guava (González-Aguilar et al., 2004), mango (Junmatong et al., 2012) or tomato (Zhang et al., 2011; Aghdam et al., 2012). In tomatoes, Ding et al. (2002) reported that 0.01 mM MeSA (methyl salicylate) and MeJA (methyl jasmonate) vapour treatment increased chilling tolerance and decreased decay. Similarly, MeJA application (10 $\mu mol.L^{-1}$) inhibited green mould decay and reduced CI symptoms in grapefruit during storage at 2 °C and subsequent post-storage period at 20 °C (Droby et al., 1999). Meir et al. (1996) suggested that SA is endogenously synthesised, playing an essential role in thermogenesis and induction of several defence responses. Several triazoles such as paclobutrazol also increased tolerance to CI in seedlings of bean and zucchini squash (Lee et al., 1985).

1.4.6. Calcium and other chemicals

There have been some good correlations between calcium content in tissues and susceptibility of fruit and vegetables to CI. For example, lime fruit with the lowest calcium content in their juice developed the highest percentage of CI (Slutzky et al., 1981). Similarly, application of calcium significantly reduced severity of CI in avocado, okra, peach, and tomato (Wang, 2010).

Some chemicals that possess properties of antioxidants, free radical scavengers or plant protection fungicide have been reported to reduce CI in many crops. Cucumber and sweet pepper treated with ethoxyquin and sodium benzoate maintained a high degree of unsaturation of fatty acids and showed a reduction of CI (Wang and Baker, 1979). Jones et al. (1978) indicated that postharvest treatment with dimethylpolysiloxane, safflower oil, or mineral oil prevented chilling-induced underpeel discolouration of bananas. Hordijk et al. (2012) suggested that Navel oranges ('Autumn Gold' and 'Cambria') and 'Star Ruby' grapefruit treated with thiabendazole (Tecto®, a fungicide) at 40 mL/20 L in warm water (45 °C) showed reduced CI. Alleviation of CI in lemon by 1 or 10 μM molybdenum (Mo) dips in hot water (53 °C) was also reported (Mathaba et al., 2012).

The use of 1-MCP influencing chilling sensitivity has been discussed in this review (section 1.2.1.2.2). 1-MCP is a cyclic olefin that binds competitively and irreversibly to ethylene receptors (Sisler et al., 1996). 1-MCP is very effective and stable, non-toxic, and most importantly, has no harmful effects on environment (Lurie and Paliyath, 2008). Both positive and negative effects of 1-MCP application on chilling sensitivity have been reported. 1-MCP has been found to reduce CI in melon (Ben-Amor et al., 1999), avocado (Pesis et al., 2002), persimmon (Salvador et al., 2004), pineapple (Selvarajah et al., 2001), loquat (Cai et al., 2006a), plum (Candan et al., 2008), pear (Argenta et al., 2003), and apple (Fan et al., 1999; Zanella, 2003).

In contrast, application of 1-MCP increased CI in some crops including peaches (Girardi et al., 2005), nectarines (Dong et al., 2001), bananas (Jiang et al., 2004), and tomatoes (Jing and Zi-Sheng, 2011). Importantly, ethylene can alleviate the CI problem in these crops, indicating that beneficial or detrimental effect of 1-MCP in influencing CI is more likely dependent on sensitivity of tissues to ethylene. However, in some tissues (e.g. 'Shamouti' oranges), ethylene markedly enhanced the appearance of CI symptoms, stem-end rot, and

off-flavour but inhibiting the ethylene action by 1-MCP did not reverse the ethylene response, instead increasing the CI symptoms, decay development and accumulation of volatile off-flavours (Porat et al., 1999). This clearly indicates that the role of ethylene or 1-MCP in influencing CI is not always straightforward dependence on ethylene sensitivity and may be dependent on other attributes of fruit tissues.

Overall, treatment with 1-MCP usually enhances resistance to CI in some horticultural crops, but in some cases it does not have any positive effect instead renders fruit tissue increased susceptibility to CI. Since chilling sensitivity in some crops (e.g. tomato) usually reduces once the fruit advances to full ripeness, it is possible that 1-MCP increases chilling sensitivity by delaying ripening. On the other hand, since in some crops ethylene enhances chilling sensitivity, elimination of ethylene response by 1-MCP reduces CI. Therefore, the physiological basis for induction or prevention of chilling injury in response to 1-MCP may largely depend on ethylene sensitivity of that particular symptom of tissue (Watkins, 2006). Variation in application dose, period of application, crop species or ripening stage may explain these discrepancies. Additionally, ability of fruit tissue to synthesise new receptors and recover their sensitivity to ethylene (Sisler and Serek, 1997) may need to be considered and thus more than one application may be required.

1.5. IW and possible mechanisms to reduce CI

The physiological abnormality or dysfunction of commodities due to low, non-freezing temperature can be reduced or delayed by manipulating temperature from low to high and then back to low one or more times for various periods of time (Hatton, 1990). Although studies show that IW can be very useful in preventing chilling injury and improving quality of fruit and vegetables, the greatest difficulty lies in practical logistical difficulties associated with warming and cooling large volumes of fresh produce. Determining optimum storage conditions for IW storage that will favour commercial adoption is challenging. Researchers have been trying to manipulate various intermittent warming regimes in various crops to reduce CI for a long time. The optimum regime varies from cultivar to cultivar and with fruit maturity stages and growing conditions. This review reports some of the temperature-time matrices employed to reduce CI in horticultural crops. The section also attempts to elucidate the possible mechanisms of how IW reduces

CI in crops. The understanding of mechanisms may allow us to identify a novel technique which may harness the benefit of IW without logistical problems.

1.5.1. Reduction of chilling injury by IW

Intermittent warming has been shown to be effective in reducing CI in many horticultural crops (Table 1.1). IW also shows potential to improve or retain fruit quality during postharvest storage. For instance, pomegranate fruit warmed intermittently at 20 °C for 1 d after every 6 d at 2 or 5 °C storage showed highest anthocyanin concentration and titratable acidity content and best visual appearance compared to fruit stored uninterruptedly in cool storage (Artés et al., 2000). Beneficial effect of IW on market quality also includes increased TSS/TA ratio (Fernández-Trujillo and Artés, 1997a) or increased vitamin C (Ruoyi et al., 2005) in peaches, and inhibition of green colour loss in limes (Kluge et al., 2003). In addition, IW at 20 °C for 1 d every week alleviated the loss of aroma-related ester by regulating ATT activity in peaches stored at 5 °C (Xi et al., 2012). However, IW may have some detrimental effects such as IW reduced woolliness in peaches, but it encouraged decay incidence and loss of pulp firmness (Girardi et al., 2005). In 'Tahiti' lime stored at 5 °C, while IW reduced CI when warmed at 20 °C for 1 d every 14 d, the same cycle of IW to 38 °C had negative effect on quality such as fruit developed rot, lost green colour and showed reduced vitamin C (Kluge et al., 2003).

Rate of temperature change, extent of temperature change, and the frequency and duration of temperature change will influence IW effectiveness in reducing CI (Wang, 1994). Three cycles of IW to 20 °C for 1 d every 7 d enhanced surface colour and reduced incidence of pitting, *Alternaria* and other decay compared with fruit that were stored continuously at 9 °C (Artés and Escriche, 1994). However, they found that the same cycle of IW was not effective in lowering CI incidence in fruit stored at 6 °C. Extent of temperature rise results in differences in effectiveness. For instance, Artés et al. (1996) found that peaches stored at 0 °C and subjected to IW to 20 °C for 1 d every 8 d alleviated CI but when the warming temperature rose to 15 °C, it did not. Internal breakdown of nectarines stored at 1 °C for 3 weeks was alleviated by warming fruit intermittently at 20 °C for 1 d per week or 2 d per fortnight; however, warming to 12 °C for 2 d per week also reduced CI (Lill, 1985). The author suggested that warming to 12 °C was likely to be more practical in a commercial situation than warming to 20 °C.

Table 1.1 Summary of IW effects in reducing CI in horticultural crops

Crop	Chilling temperature	IW regime	CI symptoms	References
Apple	0 °C	20 °C for 1 d every 1, 2, or 4 weeks	Superficial scald	Alwan and Watkins, 1999
	1 °C	20 °C for 7 d following 0, 10, 17, 31, or 58 d following storage inception	Superficial scald	Rudell et al., 2011
Cranberry	0 °C	21 °C for 1 d every 4 weeks	Physiological breakdown	Hruscha, 1970
Cucumber	2.5 °C	20 °C for 1 d every 3 d	Pitting, skin browning, decay	Wang and Baker, 1979
	2.5 °C	12.5 °C for 18 h every 3 d	Ion leakage, pitting and decay	Cabrera and Saltveit, 1990
Grapefruit	2 °C	20 °C for 1 d at the end of 1 week and again at the end of 2 weeks	Brown staining (scald), pitting	Davis & Hofmann, 1973
Lemon	2 or 8 °C	13 °C for 7 d after every 21 d	Internal membranosis, rind pitting, decay	Cohen et al., 1983
	2 °C	13 °C for 2 weeks after every 2 weeks	Decay, rind pitting, red blotch	Artés et al., 1993
Lime	5 °C	20 °C for 2 d every 7 d Or 20 °C for 2 d every 14 d	Small black superficial pit	Kluge et al., 2003; Harhas and Al-Obeed, 2006
	5 °C	15 °C for 2 d every 12 d	Pitting, brown discolouration	Pranamornkith, 2009
Okra	0 or 5 °C	20 °C for 1 d every 4 to 8 d	Overall CI symptoms	Ilker and Morris, 1975
Orange	3 °C	15 °C for 2 weeks every 3 weeks	Peel pitting, brown staining	Schirra and Cohen, 1999

Crop	Chilling temperature	IW regime	CI symptoms	References
Nectarine	1 °C	20 °C for 1 d per week or 2 d per fortnight	Internal breakdown	Lill, 1985
Peach	1 °C	20 °C for 1 or 2 d every 2 weeks	Woolliness	Buescher and Furmanski, 1978
	0 °C	20 °C for 1 d every 8 d	Woolliness	Artés et al., 1996
	0.5 °C	20 °C for 1 d every 6 d	Woolliness, decay	Fernández-Trujillo and Artés, 1997a
	2 °C	20 °C for 1 d every 6 d	Woolliness, gel breakdown and scald	Fernández-Trujillo et al., 1998
	0 °C	20 °C for 1 d on 12th day of cold storage	Woolliness	Zhou et al., 2001
	0 ± 0.5 °C	20 ± 3 °C for 1 d on 15th d of storage	Woolliness but encouraged fruit decay and loss in pulp firmness	Girardi et al., 2005
	1 ± 0.5 °C	22 °C for 1 d every 24 d (in combination with 0.05 g $CaCl_2$ + 1.0 g chitosan/100 mL)	Internal browning	Ruoyi et al., 2005
	5 °C	20 °C for 1 d every week	Internal browning	Xi et al., 2012
Plum	0 °C	18 °C for 2 d between 15th and 20th d	Internal browning	Smith, 1947
	0 °C	20 °C for 1 d every 10 or 15 d (combined with MAP)	Brown surface, flesh browning, ion leakage	Ding et al., 2010

Crop	Chilling temperature	IW regime	CI symptoms	References
Pomegranate	2 or 5 °C	20 °C for 1 d every 6 d	Surface pitting, husk scald	Artés et al., 2000
Potato	0 °C	16 °C for 1 week after every 3rd week	Browning, blackheart, bluish skin discolouration, surface mould	Hruscha et al., 1969
Sweet pepper	2.5 °C	20 °C for 1 d every 3 d	Pitting, skin browning, decay	Wang and Baker, 1979
Tomato	6 or 9 °C	20 °C for 1 d every 7 d	Abnormal red colour, pitting, *Alternaria*	Artés and Escriche, 1994; Artés et al., 1998b
	9 °C	20 °C for 1 d every week	Pitting, *Alternaria*	Artés et al., 1998a
Zucchini Squash	2.5 °C	20 °C for 1 d every 2 d	Surface pitting	Kramer and Wang, 1989

In summary, periodic interruptions of low temperature with one or more short periods of warm temperature alleviate CI during low temperature storage of many fruit and vegetables. IW also shows promise for extending storage life and improving market quality of some commodities. However, the optimal frequency, duration of warm temperature and the extent of temperature change vary from crop to crop. Since pre-harvest growing conditions influence CI, it is likely that effectiveness of IW in reducing CI will depend on those conditions. Importantly, IW must be applied before CI becomes irreversible (Wang, 1993). If the critical time at chilling temperature has been exceeded and CI progresses beyond the point of recovery then raising the temperature would only accelerate the degrading process and hasten development of CI symptoms. Conversely too early or too frequent warming periods can result in excessive softening and vulnerability to pathogens (Wang, 1993). Therefore, correct timing and duration is essential for successful IW treatments.

1.5.2. Possible mechanisms of IW in prevention of CI

The mechanism(s) by which IW reduces CI is not clear (Sevillano et al., 2009). Intermittent warming is hypothesised to restore metabolite concentrations that were disturbed during cool storage. Restoration of metabolites by IW can be done in a number of ways: (i) IW allows tissues to metabolise excess intermediates accumulated during the cold cycle or (ii) allows tissues to restore any substances that were depleted or (iii) allows tissues to synthesis the new compounds which were not able to be made during the chilling period (Wang, 1990).

1.5.2.1. IW allows metabolising excess intermediates

One of the earliest theories related to the cause of chilling injury proposed that the accumulation of toxic compounds was caused by a temperature-induced imbalance in metabolism (Nelson, 1926 as cited in Saltveit and Morris, 1990). Nelson suggested that surface pitting was associated with the products of abnormal respiration. IW helped to remove toxic or inhibiting substances that accumulated during chilling (Penter and Heinze, 1954) or higher metabolic activity due to IW replenished deficiencies that developed during chilling (Wang, 1993). Reduction of superficial scald in cool-stored apple by intermittent warming could be associated with either inhibition of toxic compounds such as α-farnesene and its oxidation products or enhanced catabolism of conjugated trienes during

warming periods (Alwan and Watkins, 1999; Rudell et al., 2011). In this way mode of action of IW in inhibiting superficial scald might be quite similar to heat treatments. Heat treatments of apples inhibited synthesis of α-farnesene and therefore oxidation and accumulation of conjugated trienes, which causes cellular damage resulting in superficial scald (Lurie et al., 1990). However, scald can reappear in heat-treated apple at low storage temperatures as synthesis of α-farnesene gradually recovers (Lurie et al., 1990; Combrink et al., 1994). In that case, cycles of IW may be better than one-time pre-storage heat treatments to inhibit α-farnesene synthesis and reduce subsequent scald.

Internal browning of peaches can be reduced by the combination of chitosan, $CaCl_2$ and IW by inhibiting PPO (polyphenol oxidase) and POD (peroxidase) activity (Ruoyi et al., 2005). Generally, PPO catalyses the oxidation of O-diphenols, thus producing O-quinones and polymerisation of O-quinones produces brown pigments (Ruoyi et al., 2005).

1.5.2.2. IW allows the tissues to restore substances that were depleted

1.5.2.2.1. Heat treatments and lipid composition in cell membrane

Chilling injury has long been thought to begin with membrane damage (Lyons, 1973). Temperature plays an important role in regulating the lipid composition of cell membranes and ultimately membrane fluidity (Murata and Nishida, 1990). Cell membranes of fruit exposed to higher temperature remain more fluid at low temperature due to a higher proportion of unsaturated fatty acids (Mikami and Murata, 2003). Although temperature may have an impact on membrane fluidity at the cellular and molecular level (Nishida and Murata, 1996), it is unclear whether greater membrane fluidity developing because of a higher temperature was a direct effect of temperature or an indirect effect of altered lipid composition. Pre-storage heat treatment inhibited loss of phospholipids and led to a lower rate of K^+ leakage during cold storage whereas unheated tomatoes had a large increase in leakage and developed brown tissue areas symptomatic of CI in tomatoes at 2 °C for 3 weeks (Lurie and Klein, 1991). Saltveit (1991) found that conditioning tomato discs at 37 °C for 4 h reduced leakage indicating less cellular membrane damage. Higher degree of fatty acid unsaturation was also reported in heat-treated apples than non-heated fruit (Lurie et al., 1995). Prior warming before low temperature may help the crop to avoid gel formation as a result of increased unsaturated fatty acids content that may help to keep cellular membranes fluid and therefore alleviate CI (Wang, 1993). However, an increased

concentration of unsaturated fatty acids following high temperature conditioning affecting chilling resistance is not universal. No relationship was found between the increased unsaturated fatty acids in flavedo tissue and expression of CI in 'Olinda' oranges (Schirra and Cohen, 1999), who indicated that these increases may be the result of an unspecified fruit response to low temperature and may not be causally related to altered fruit resistance to CI.

An increase in concentrations of unsaturated fatty acids was also found during IW (Wang and Baker, 1979). Synthesis of unsaturated fatty acids during IW may result from induced elongation of fatty acids during warming and desaturation of fatty acids during cooling (Wang, 1982). Rapid changes in desaturase enzyme activity and lipid composition also occurred with altered temperatures. Wada and Murata (1990) reported that glycerolipids and fatty acid composition of *Cyanobacterium* changed with alteration in temperature and desaturase activity was enhanced after shifting from a higher to a lower temperature.

Since intermittent warming could maintain high level of phospholipids and increase degree of unsaturation of fatty acid concentrations during a rapid readjustment of metabolism (Wang, 1982), IW probably helps the membrane to return the lipids to a less gel-forming condition and undoes the primary response. Consequently, IW may allow repair of damaged membranes and increased resistance to CI. Niki et al. (1979) found some recovery of rough endoplasmic reticulum and some development of polysomes when cultured cells of *Cornus stolonifera* were re-warmed at 26 °C during chilling at 0 °C. Since heat-treated fruit had more fluid membrane due to a higher proportion of unsaturated fatty acid and lower sterol to phospholipids ratio (Lurie et al., 1997), it is possible that an increase in concentration of unsaturated fatty acids in intermittently warmed fruit may be an important factor affecting chilling resistance.

1.5.2.3. IW allows synthesis of new compounds

1.5.2.3.1. Polyamines

IW may help tissues to synthesise new compounds during a warming period. IW increased the concentrations of polyamines (spermidine and spermine) and stimulated activities of free radical scavenging enzymes in cucumber and pepper fruit (Wang and Baker, 1979). A number of studies found that heat treatment elevated the concentration of polyamines that

can stabilise membranes (Rodov et al., 1995; Valero et al., 1998). Polyamines are able to bind to negatively charged molecules as biologically essential as phospholipids, proteins and nucleic acids because of their polycationic nature (Smith, 1985). For instance, Roberts et al. (1986) indicated that the bindings of polyamines with anionic groups of membrane phospholipids could stabilise cell membranes under stress conditions and subsequently delayed membrane disintegration. Polyamines also have antioxidant properties. Therefore, binding phospholipids and removing free radical species, polyamines could protect cell membranes against lipid peroxidation, one of the main symptoms of most abiotic stresses, and preserve membrane integrity (Serrano et al., 1996).

1.5.2.3.2. Role of antioxidants against reactive oxygen species

Many stresses, including low temperature, cause cellular damage that results from perturbations in the antioxidant system in plant tissues, resulting in oxidative damage (Wismer, 2003). Oxidative stress leading to cellular damage is considered to be an early response of sensitive tissues to chilling (Parkin and Kuo, 1989). The induced ROS react with different cell components and cause a cascade of oxidative reactions including lipid peroxidation, protein degradation, and DNA damage (Scandalios, 1993). Lipid peroxidation-associated degradative processes of membranes are normal metabolic events occurring in both stressed and un-stressed tissue. While in unstressed tissue, biosynthetic defence system and repair mechanisms prevent degradation from leading to injury, in stressed situation, defence system is compromised or increase in degradative reaction exceeds the capacity of defence mechanisms (Purvis and Shewfelt, 1993).

Fruit and vegetables are considered a rich source of antioxidants. These antioxidants have an influence on the removal of these ROS (peroxy radicals (ROO), superoxide radicals (O_2) and other molecules) (Scandalios, 1993). This system involves lipid soluble antioxidants (tocopherol and carotenoids), water soluble reductants (glutathione and ascorbate) and active oxygen scavenging enzymes, including superoxide dismutase, catalase, peroxidase, and glutathione reductase (Malacrida et al., 2006). Free radical scavengers like sodium benzoate or ethoxyquine prevented oxidative destruction of unsaturated fatty acids in cell membrane and reduced severity of CI of cucumber and pepper during cool storage (Wang and Baker, 1979).

Antioxidant concentration and activity is thought to be related to storage temperature. Low temperature storage decreased both phenolic content, lycopene content and free radical scavenging capacity (Ancos et al., 2000; Javanmardi and Kubota, 2006). On the other hand, anthocyanin, phenolic, flavonoid concentrations increased at higher temperature and antioxidant activity was greater at higher than at lower temperature (Ayala-Zavala et al., 2004; Shin et al., 2008). An increase in total antioxidant concentrations and activity was found in heat-treated tomatoes (Sozzi et al., 1996), mandarins (Sala and Lafuente, 1999) or in apples (Shaham et al., 2003). However, it was unclear whether the increased antioxidant concentration and activity due to heat treatments were independent of ripening.

A positive correlation between total phenolic compounds and antioxidant activity and increased resistance to chilling stress in tomato has been reported (Senaratna et al., 1988). Since IW stimulates synthesis of antioxidant compounds (polyamines) and other heat treatments enhance antioxidant activity and concentration, IW treatments probably reduce CI by enhancing antioxidant protection mechanisms and thereby protecting fruit tissue from oxidative stress during low temperature storage.

1.5.2.3.3. Heat shock proteins

No information could be found on the synthesis of HSPs in response to intermittent warming. Rate of warming and extent of temperature change are important for induction of HSPs (Lafuente et al., 1991). Alleviation of CI and induced chilling tolerance by heat treatments usually involves exposure of plant tissues to a relatively higher temperature (around 40-50 °C) than the temperature employed during intermittent warming (around 20 °C). Therefore, it is unclear whether that extent of temperature rise during IW period is enough to induce HSPs. Lafuente et al. (1991) indicated that exposure of plant tissues to a sudden jump of temperature about 5 - 10 °C above the normal growing temperature may well be enough to induce the synthesis of these HSPs. Therefore, it is possible that warming fruit intermittently during exposure to low temperatures may stimulate synthesis of HSPs which will be involved in recovery from chilling stress and confer protection for subsequent chilling stress.

Generally HSPs are not stable at ambient temperatures, but are metabolised and disappear from tissue within a few days of stress (Lurie, 2005). However, Ilic and Fallik (2005)

indicated that protection afforded by heat shock against chilling injury in tomato persisted up to 21 d at 2 °C. If IW treatment is able to induce HSPs, repeated warming (generally more than one cycle involve in case of IW) may provide long term protection, better than what one time heat treatment may do.

1.5.2.3.4. Ethylene and ethylene mediated enzymes

Endogenous ethylene produced following exposure to IW enabled cold stored peaches to ripen normally and avoid woolliness (Fernández-Trujillo and Artés, 1997a). During cold storage of peaches, mRNA of ACS and ACO decreased thus ACC failed to convert to ethylene (Zhou et al., 2001). Accumulation of ACC was detected in chilled cucumber tissues stored at 2.5 °C (Wang and Adams, 1980). Significantly lower ACO activities were found in chilled cucumber stored at 2.5 °C (Cabrera and Saltveit, 1990), whereas warming of cucumber stored at 4 °C to 14 °C after 16 and 18 d of chilling resulted in increased accumulation of both ACC and ACS activities (Kuo and Parkin, 1989). Similarly in peaches, while chilling temperature inhibited the reaction that converted ACC to ethylene, warming fruit intermittently resulted in induction of ACO mRNA abundance and stimulation of conversion of ACC to ethylene (Zhou et al., 2001).

Tomato is a climacteric fruit and it has been reported that continuous production and action of ethylene are required for integration of the biochemical processes of ripening (Theologis, 1992; Hoeberichts et al., 2002). With respect to the reduction of woolliness in peaches and nectarines, it was hypothesised that raising the temperature temporarily would prevent accumulation of ACC and help convert ACC to ethylene and maintain activity of enzymes in the sequence of cell wall hydrolysis necessary for normal ripening (Zhou et al., 2001). This suggestion might also be true for tomato. IW stimulated ethylene production in tomato and advanced colour development during low temperature storage (Artés et al., 1998a). Since failure to achieve normal red colouration or uneven blotchy red colouration have been shown to be the main CI symptoms in tomatoes, it is possible that IW stimulates ethylene production and enables fruit to develop red colour and ripen normally. Generally chilling sensitivity in tomato reduces as fruit ripen (Autio and Bramlage, 1986) and this could be related to advancement of climacteric ethylene. Other postharvest heat treatments such as hot water treatment increased ethylene evolution and this ethylene production was correlated with enhanced red colour development (McDonald et al., 1999; Soto-Zamora et

al., 2005). However, it remains unclear whether advanced ripening by heat treatment is a cause or consequence of stimulated ethylene production.

1.6. Aims and project objectives

This chapter has reviewed the CI phenomenon in tomato and the methods used to alleviate this disorder. CI can be reduced by several methods including temperature conditioning, intermittent warming, controlled or modified atmosphere, and application of growth regulators or other chemicals. Among the different postharvest technologies available to prevent or alleviate CI, it was decided to work with intermittent warming as it is a technique with much potential to alleviate CI in many horticultural crops. Low temperature storage is essential to extend postharvest life of many crops, but after a certain period of time in a particular temperature, chilling-sensitive crops develop CI. To prevent that problem, it would be beneficial to remove the chilling stress occasionally before it reaches the irreversible phase and move products to a higher temperature for a certain period before they are returned to chilling conditions. Therefore, in theory, the concept of IW is an ideal technique in every sense to reduce CI.

Tomato is one of the most extensively studied crops and physiological, biochemical and molecular aspects of tomato ripening have been studied over a long period for various research purposes because of the existence of well characterised developmental mutants and ease of genetic manipulation (Alexander and Grierson, 2002). In addition, relatively short life cycle and economic importance as a crop are important factors in the selection of this crop for this study.

Tomato is susceptible to low temperature storage below 13 °C. Information in the literature suggested that time and temperature combination along with cultivar, maturity, and pre-harvest growing conditions influence chilling sensitivity and subsequent development of CI symptoms. Therefore, to study the CI phenomenon, it is necessary to characterise CI symptoms of a specific cultivar grown in a particular condition. As CI is a set of physiological damage symptoms below a threshold chilling temperature, it is possible that induction of a particular injury symptom needs a particular time-temperature combination. Therefore, it is also important to determine whether different CI symptoms of the tomatoes studied require different threshold temperatures to display damage.

Mechanisms of many chilling-induced physiological alterations can be confounded with normal ripening-associated changes since some of the processes occurring during the development of CI are similar to those which occur during ripening and senescence. Therefore, it is important to differentiate the physiological or cellular alteration accompanying ripening from the changes that reflect chilling damage. Low temperature storage usually delayed ripening and in literature sometimes slow ripening or delayed ripening is interpreted as symptomatic of CI. Therefore, it is important to distinguish CI symptoms from a simple delay in ripening caused by non-damaging low temperature storage.

Intermittent warming can reduce CI in tomato. Despite the positive results of IW in preventing chilling injury and improving quality, there are practical difficulties involved in warming large volumes of fresh produce. Repeatedly increasing and decreasing storage temperature is usually a slow process that is expensive to achieve quickly (e.g. by forced draft heating or cooling). Additionally, condensation problems during warming and cooling cycles may lead to postharvest rots. The optimum regime may change from cultivar to cultivar, with fruit maturity stage and growing conditions. Importantly, the successful IW regime is arrived at empirically, by trial and error, and what works on one cultivar or its growing condition may not be as successful on another. Therefore, the present study was undertaken to elucidate a basic physiological responses by which IW reduces CI in tomato instead of trying to optimise another successful regime for a cultivar.

Intermittent warming reduces woolliness in peaches by enhancing ethylene production and enzymes mediated by ethylene. Similarly, IW and other heat treatments stimulate ethylene production and advance red colour development in tomatoes. Since altered red colour development is the main CI symptom and ethylene is a regulator for normal red colouration, it is plausible that IW-stimulated ethylene causes a reduction of CI, determined by altered red colouration in tomato.

A deeper understanding of the physiological responses by which IW exerts its beneficial effects may allow us to identify novel techniques that will harness the benefits of IW without the logistical problems that are associated with industrial application. Additionally, this understanding may allow us to develop mathematical models in order to optimise the intermittent warming regime in horticultural crops in future research or it may help to

suggest a novel technique which is more practical and may be quite different from IW. From a science point of view, an understanding of the influence of IW on chilling injury could provide information about the physiological and biochemical basis of the disorder.

Therefore, the overall objectives of this study are:

- To characterise the chilling injury symptoms of tomatoes ('Cedrico') grown in New Zealand glasshouse
- To distinguish delayed ripening from true CI
- To determine the effect of IW in reducing CI of 'Cedrico' tomatoes
- To investigate the physiological responses by which IW reduces CI in tomato including the potential role of IW-stimulated ethylene
- To determine if there is any role of ethylene in reducing CI independently of increased temperature (IW) by carrying out application of exogenous ethylene and inhibition of its effect by use of 1-MCP.

2. Characterisation of tomato chilling injury symptoms (*)

2.1. Introduction

Tomatoes are susceptible to chilling injury when stored at temperatures below 13 °C for 2 weeks or longer (Efiuvwevwere and Thorne, 1988). CI symptoms in tomatoes can include failure to ripen properly, pitting, shrivel, water soaking, increased susceptibility to decay and altered aroma profile (Hobson, 1981; Efiuvwevwere and Thorne, 1988; Maul et al., 2000). CI symptoms generally do not appear until after tomatoes are transferred to non-chilling temperatures (Morris, 1982). A strong stimulation of ethylene biosynthesis especially upon post-chilling removal to warmer temperatures was often correlated with the appearance of CI symptoms in tomato (Cheng and Shewfelt, 1988). However, no consistent relationship between ethylene production and CI has been reported.

The rate of development and magnitude of the visible CI symptoms in tomatoes vary with fruit maturity. Generally, mature-green tomatoes are more sensitive to low temperature than ripe tomatoes (Autio and Bramlage, 1986). Saltveit and Morris (1990), however, argued that ripe fruit may appear more chilling-resistant simply because they cannot exhibit alterations in the already accomplished process of ripening (i.e. red colour development). Secondly, the degree of CI severity is a function of storage temperature and time interaction (Hobson, 1981; Saltveit and Morris, 1990). Finally, genotype and pre-harvest growing conditions also influence the chilling sensitivity in tomato (Abou-Aziz et al., 1974; Saltveit, 1991).

Cheng and Shewfelt (1988) reported that mature-green 'Flora-Dade' tomatoes stored at 4 °C showed a pronounced increase in decay after 15 d storage and irreversible inhibition of colour development after 34 d. Israel-grown 'Rehovot 121' tomatoes held at 2 °C for 21 d remained green and developed CI (Lurie and Klein, 1992), whereas only 14 d at that temperature resulted in the inhibition of red colour development in Florida-grown 'Sunbeam' tomatoes (McDonald et al., 1999). Chomchalow et al. (2002) indicated that exposure of mature-green 'Sunny' tomatoes to 2.5 °C for as little as 3 d caused blotchy ripening and decay. Inhibition of red colour development was also reported for breaker tomatoes stored at 3-6 °C for 2 to 4 weeks (Gómez et al., 2009; Rugkong et al., 2011).

(*) Material from this chapter is included in the paper: Biswas, P., East, A. R., Brecht, J. K., Hewett, E. W., & Heyes, J. A. (2012a). Intermittent warming during low temperature storage reduces tomato chilling injury. *Postharvest Biology and Technology*, *74*, 71-78.

Moline (1976) found that Florida-grown 'Walter' tomatoes stored at 2 or 7 °C for 10 d lost the capacity to convert chloroplasts to chromoplasts and after 15 d fruit had swollen and degenerated mitochondria and plastids. A storage temperature below 15 °C for 7 d impaired California-grown 'Cal Ace' tomato flavour development (Kader et al., 1978), while storage below 12.5 °C affected flavour of Florida-grown 'BHN-189' and 'Solimar' tomatoes after just 2-4 d (Maul et al., 2000). Therefore, results reported here suggest that not only do time and temperature combinations influence CI, but other factors such as production conditions and cultivar are also likely to have an important influence (Saltveit, 2005). Additionally, Hobson (1987) demonstrated that different chilling conditions may result in varied types of symptoms (Table 2.1).

Table 2.1 Various degrees of chilling injury damage of breaker tomatoes ('Sonatine') upon ripening in air at 20 °C following chilling at different temperatures for different period of time (Hobson, 1987).

Inducing treatment	Extent of damage	Response
Sealed in polyethylene for 11 d at 5 °C and then transferred to 20 °C. Alternatively, 5 °C for 9 d without protection	Severe	Large, firm, extensive green patches on otherwise red fruit. Surface contours uneven due to cell collapse, or in extreme cases, desiccation and wrinkling of the fruit
Stored at 5 - 7.5 °C for 9 d in air	Moderate	Uneven surface, with mottled yellow areas on a red background. Damage sometimes takes the form of translucent water-soaked patches on an otherwise red fruit
Stored in double 'Clingwrap' at 7.5 - 8.5 °C for 6 d	Intermediate	Small surface-depressions and unevenness, accompanied by failure to pigment evenly
Stored at 7.5 - 8.5 °C for 6 d in air. Enclosure for 6 d or more at 20 °C under conditions fostering excessive build-up of CO_2 and/or ethylene	Slight	The first sign of chilling injury is a loss of firmness by fruit. Additionally such fruit may also show non-uniform colouration especially between the lines where the inner locule walls meet the outer ones. Only in mild cases does the condition ripen completely

Since the physical and physiological damage that occurs during chilling injury can be varied with tomato genotype and pre-harvest growing conditions, the goal of this chapter is to characterise the CI symptoms of the cultivars used in this work. The chilling responses of 'Cedrico' (grown in a greenhouse in New Zealand) are compared with 'Soraya' grown outdoors in Florida. This work also aimed to move the research away from using collective term of "chilling injury" by differentiating CI symptoms of the tomatoes studied as influenced by different temperatures.

2.2. Materials and methods

2.2.1. Plant material and storage conditions

New Zealand-grown 'Cedrico' tomatoes were grown in a greenhouse (Massey University Plant Growth Unit, Palmerston North) during the early summer season using standard glasshouse production practices. Potting mix included dolomite, 8-9 months osmocote with Dalton base mix (Calcium Ammonium Nitrate: Fibre: Pacific Pumice – 5:3:2) and a pH of 5.3 was maintained. Every day plants were irrigated twice (1 h after sunrise and 1.5 to 2 h before sunset). Length of each irrigation was constant and expected to give a drain of approximately 20%. Nutrients were injected via drip irrigation (fertigation) into the pots from concentrated solution in stock tanks. There were 2 solutions which were stock solution A (calcium nitrate 19.80 kg + potassium nitrate 13.16 kg in 200 L water) and stock solution B (magnesium sulphate 9.94 kg + mono potassium phosphate 5.4 kg + iron chelate 600 g + manganous sulphate 100 g + zinc sulphate 7 g + copper sulphate 6 g + boric acid 36 g + ammonium molybdate 1.6 g in 200 L water). The medium solution A:B (1:1) was diluted with water 1: 1000. All plants were sprayed approximately once every fortnight with Attack^{TM} (pyrethroid and organophosphate), Chess^{TM} (pyridine azomethine), and Nuvos^{TM} (dichlorvos), each time with alternate pesticide. On some occasions Chess and dithane (0.4 g/L & 1.5 g/L) or Attack and thiram (1.0 ml/L & 1.5 g/L) were sprayed in combination. Whitefly and aphids were the only pests found in the glasshouse. Average, maximum, and minimum temperatures in the greenhouse were maintained at 20, 25, and 16 °C respectively, with an average day length of 15 h during fruit development (30 d prior to harvest). Temperatures were logged in a shaded box with a fan to allow air movement. Fruit were harvested at the mature-green stage and brought immediately to the laboratory. Fruit free from external defects and of a uniform size were selected and left at 20 °C over night. The fruit were not washed or treated with any fungicide after harvest. A total of 45

fruit were randomly grouped into three replicate batches of 15 fruit for each treatment. Next day after harvest, fruit calyces were removed carefully prior to packing into cardboard trays containing a plixtray and single-layer polyethylene liner. The trays were placed on wire racks without stacking. Fruit were held at 2.5, 6, 8, or 20 °C and with 90-95% relative humidity for 27 d. Additionally, some fruit were transferred to 20 °C following 13 d of storage and only decay was evaluated subsequently after 7 d. Fruit physiology (ethylene production) and quality (colour, firmness, and decay development) were evaluated during 27 d of storage and a subsequent 7 d post-chilling period at 20 °C.

In Florida, mature-green tomatoes ('Soraya') were grown in an open field. Tomatoes were grown in southwest Florida during the spring season using standard commercial production practices including soil fumigation with methyl bromide and chloropicrin, raised beds with plastic mulch, seepage irrigation, and a plant population of 9,800 ha^{-1}. Temperature conditions during fruit development (30 d prior to harvest) were 26 (average), 32 (maximum) and 21 °C (minimum), respectively, with 42 mm total rainfall and average day length of 12 h. Fruit were obtained from a local commercial packhouse near Tampa, Florida and transported by air-conditioned vehicle 4 h to the Postharvest Laboratory at the Horticultural Sciences Department, University of Florida, Gainesville. Upon arrival, fruit were kept at 20 °C until the next morning (15 h). Fruit of uniform size and free from defects and blemishes were randomised into 54-fruit lots in three replicates of 18 fruit for each treatment. Six fruit of each replicate were placed in a plastic box on a commercial Styrofoam packaging tray. The trays with tomatoes were placed on a storage rack that was covered with transparent zip-lock plastic floating over the top to avoid excess air movement. Fruit were held at 2.5, 6, 12.5, or 20 °C, with 90-95% RH for 27 d. To investigate whether ripe tomatoes are less chilling sensitive than mature-green fruit, some fruit at pink maturity stage were also stored at 2.5 or 6 °C for 13 d and CI (as determined by decay susceptibility) was subsequently evaluated after 7 d post-storage period at 20 °C.

2.2.2. Surface colour

Fruit colour was planned to be evaluated during 27 d of storage and a subsequent 7 d post-chilling period at 20 °C. Surface colour of New Zealand-grown tomatoes was measured with a reflectance spectrophotometer (CM-2600D, Konica Minolta Sensing Inc., Japan) and for Florida-grown tomatoes with a hand-held tristimulus reflectance colorimeter

(Model CR-200b, Minolta Corp., N.J., USA) on each fruit surface at three locations around the fruit equator. In New Zealand 15 fruit were measured while in Florida 18 fruit were measured on each occasion for each treatment. Average lightness (L^*), chroma (C*) and hue angle (h°) of the three locations were calculated. After 27 d Florida-grown 'Soraya' tomatoes stored at 2.5 or 6 °C exhibited excessive rot, so no colour data were recorded for these fruit.

2.2.3. Ethylene production

For New Zealand-grown tomatoes, individual fruit were sealed in a 578 mL jar equipped with a rubber septum to enable gas sampling at the storage temperature. A 1 mL gas sample was collected shortly after sealing the jar and subsequently after a known time. Ethylene concentration of the sample was measured with a Varian 3400 gas chromatograph (GC) fitted with a flame ionization detector and equipped with a 3.2 mm diameter alumina column (AllTech Associates, New Zealand). The column was set at 90 °C with N_2 as the carrier gas at a flow rate of 35 $mL.min^{-1}$. Flame ionization detector temperature was set at 120 °C with H_2 and air flow rates of 20 and 300 $mL.min^{-1}$ respectively. A β-standard gas (10.3 ± 0.2 $\mu L.L^{-1}$ ethylene in air) was used for calibration (BOC, New Zealand). Output signals were analysed and recorded with an HP 3394A (Hewlett Packard, USA) integrator.

For the Florida experiment, a batch of six fruit was sealed in a 3 L jar equipped with a rubber septum to enable gas sampling at the storage temperature. A 3 mL gas sample was collected shortly after sealing the jar and subsequently after a known time. Ethylene concentration of the sample was determined using a GC fitted with a pulse discharge helium ionization detector (Model CP3800, Varian Instruments, Palo Alto, CA, USA). The Hayesep Q 80/100 0.5 m x 3.2 mm ultimetal column was set at 50 °C with ultra pure helium as the carrier gas at a flow rate of 20 $mL.min^{-1}$. Injector and detector temperature were set at 220 and 120 °C respectively. Calibration was done using a standard sample gas (0.98 $\mu L.L^{-1}$ C_2H_4, 1.02% CO_2, 20.01% O_2, balance nitrogen, certified standard, Airgas Inc., PA, USA).

Rate of ethylene production ($mol.kg^{-1}.s^{-1}$) was calculated using following equation:

$$\text{Production Rate} = \frac{\left(V_C - \frac{m}{\rho}\right)(C_1 - C_0)P \times 10^{-6}}{R.T.m.t}$$

V_C = jar volume (m^3); m = fruit mass (kg); ρ = density ($kg.m^{-3}$); C_0 = initial concentration ($\mu L.L^{-1}$); C_1 = concentration at time t ($\mu L.L^{-1}$); P = atmospheric pressure (Pa); R = universal gas constant (8.314 $Pa.m^3.mol^{-1}K^{-1}$); T = temperature (K); t = time between samples (s), 10^{-6} = conversion factor ($\mu L.L^{-1}$ to mol fraction).

2.2.4. Fruit firmness

Fruit firmness was measured only for the tomatoes grown in New Zealand. Firmness of 15 fruit on each occasion for each treatment was measured non-destructively using two methods – acoustic firmness (Aweta BV, Nootdorp, The Netherlands) and compression firmness (TA-XT Plus, Stable Microsystem Ltd., USA). Both firmness tests were evaluated at the respective storage room temperatures. For acoustic firmness, a tomato was placed on the Aweta which has a cushioned ring and the fruit was impacted from beneath by a solid plastic rod (Tick length = 20 mm). A small microphone then captured the mechanical vibration (Microphone gain = 70). A stiffness index (S, $Hz^2\ kg^{2/3}$) was produced by the device based on the resonant frequency (f) of the first elliptical mode and the mass (m) of the tomato (Hertog et al., 2004). Stiffness was measured as the average of three readings on the fruit surface. Compression force readings were taken at three different locations of each fruit by recording the maximum force required to compress the fruit for 2 mm using a flat cylindrical probe (50.85 mm diameter). The compression test was run with a flat plate using a pre-test speed of 5 mm s^{-1}, a test speed of 1 mm s^{-1}, and a trigger force of 1 N.

2.2.5. Decay incidence and severity

Decay incidence was expressed as percentage of the total number of fruit manifesting decay symptoms. Decay severity was determined according to the area decayed on each fruit using a 0 to 4 visual rating scale where 0 = no decay, 1 = light - 1 to 10% decay, 2 = moderate - 11 to 30% decay, 3 = high - 31 to 60% decay, 4 = severe - greater than 60% decay. The average severity was calculated.

2.2.6. Titratable acidity and total soluble solids content

Titratable acidity (TA) and total soluble solids content (TSS) were analysed only for 'Soraya' tomatoes grown in Florida. Six tomatoes cut into halves from each replication were homogenised by a blender for 30 s and a 50-g aliquot of the tissue slurry was centrifuged at 17,600×g for 25 min at 4 °C. The clear juice was decanted from the centrifuge tubes and TA was determined using an automatic titrimeter (Metrohm Ion Analysis Ltd., model 719 S Titrino, Switzerland). Aliquots (6 mL) of tomato juice were diluted with 50 mL distilled water and the titratable acidity was determined by titration with 0.1 mol.L^{-1} sodium hydroxide (NaOH) to an end point of pH 8.2. TA was expressed as percent citric acid. The TSS of the resulting clear juice samples was determined with an Abbe refractometer (Cambridge Instruments, Inc., Buffalo, NY) and expressed as °Brix. TA and TSS were determined on day 13, 27 and 7th d at 20 °C following 13 d cool storage.

2.2.7. Volatile analysis

Volatiles were analysed only for 'Soraya' tomatoes grown in Florida on day 13, 27 and 7th d at 20 °C following 13 d cool storage (13 + 7 d 20 °C). Six tomatoes were cut into halves and homogenised for 30 s and then held for 3 min. Then 25 mL of homogenate was removed and blended for 10 s with 10 mL of saturated $CaCl_2$ (at room temperature) to inhibit enzyme activity. Duplicate aliquots (5 mL) were pipetted into 15 mL vials, which were crimp capped with PTFE/silicone septa, flash frozen in liquid N_2 and held at − 30 °C until analysis. The vials were equilibrated at 40 °C for 15 min on a rotator and volatile compounds in the headspace were adsorbed by using a solid phase micro-extraction technique (SPME) fibre (50 x 30 µm DVB/carboxen^{TM}/PDMS Stable Flex, Supelco, USA). Sampling time was 1 h. Desorption of volatile compounds trapped in the SPME fibre was carried out directly into the GC injector (1 min desorption time). During desorption, the injector was splitless mode. Volatiles were analysed using an GC Agilent-7890A (Agilent Technologies, China) equipped with a 60 m x 250 µm ID, 1 µm film thickness DB-5 capillary column (J & W 122-5563, Folsom, USA). GC was coupled with a mass spectrometer (Agilent Technologies, 5975C VLMSD with Triple Axis Detector, China). Oven temperature was initially held at 40 °C for 0.50 min and then increased to 230 °C at 4 °C/min and to 260 °C at 100 °C/min, and finally held for 11.70 min (total cycle time, including oven cool-down, approximately 60 min). Injector and detector temperatures were 200 and 280 °C, respectively.

Identification of volatiles was initially accomplished by GC-MS analysis using reference spectra in NIST98 mass-spectra library and by comparison with available authentic chemical standards. Mass spectrometer was applied as follows: Mass spectra were collected at a rate of 40/s over the mass range (m/z) of 40-450. The electron ionization energy was 70 eV, temperature of the ion source was 230 °C. Volatiles were further identified by comparison of Kovat's retention indices with those of authentic components and with published data. The Kovat's retention indices were determined using a calibration curve of an *n*-alkane series (C5-C15) injected under the same chromatographic conditions as those of the samples. Quantification of each volatile was carried out by comparing peak areas of analytes to that of nonyl acetate added at 1 μL/40 mL methyl chloride solvent as an internal standard to the tomato samples.

2.2.8. Statistical analysis

A completely randomized design was used throughout this study. Analysis of variance (ANOVA) was performed to analyse the effect of treatments using the General Linear Model procedure of SAS (SAS Institute, version 9.2, Cary, NC). When appropriate, means of different treatments were compared using Fisher's least significant difference (LSD) at the 5% level. The LSD values for repeated measures were calculated at each point of measurement or for non-repeated measures were calculated throughout the whole experiment.

For volatile analysis, aroma compound concentrations were analysed using Principal Component Analysis (PCA). ANOVA followed by Duncan's Multiple Range test (α= 0.05) was used for mean separations between treatments with SAS statistical programme.

2.3. Results and discussion

2.3.1. Surface colour

Storage temperature is an important factor in developing tomato colour (Kader, 1986). New Zealand-grown tomatoes stored at 20 °C reached full red colour within 12 d as hue angle decreased from 110° to 40° (Figure 2.1A). Red colour development was significantly slower in fruit stored at 8 °C in comparison with 20 °C-stored fruit. Fruit stored either at 2.5 or 6 °C did not develop red colour and remained green (hue > 100°). After post-chilling

transfer to 20 °C, the rate of colour change for 8 °C stored tomatoes was significantly higher ($p < 0.05$) than the fruit stored at 2.5 or 6 °C (Figure 2.1A). Importantly, fruit held at 8 °C developed full red colour (hue ≈ 50°), indicating storage at 8 °C only delayed colour development. Delayed colour development in tomato resulting from chilling has been reported by others (Cheng and Shewfelt, 1988; Sharom et al., 1994). Tomatoes held at 6 °C and subsequently moved to 20 °C developed uneven, blotchy colouration with yellow blush on the fruit shoulder. Dodds et al. (1991) suggested that decreased levels of chlorophyll in chilled tomatoes likely unveil β-carotene, causing the appearance of yellow blush colour. Chilling may also have caused accumulation of chalco-naringenin, a yellow compound found in tomato pericarp (Baker et al., 1982 as cited in Dodds et al., 1991). Fruit stored at 2.5 °C mostly failed to develop red colour during their post-chilling period at 20 °C. Instead of delayed colour change (as observed at 8 °C), tomatoes stored ≤ 6 °C appeared to have lost the capacity for normal red colour development.

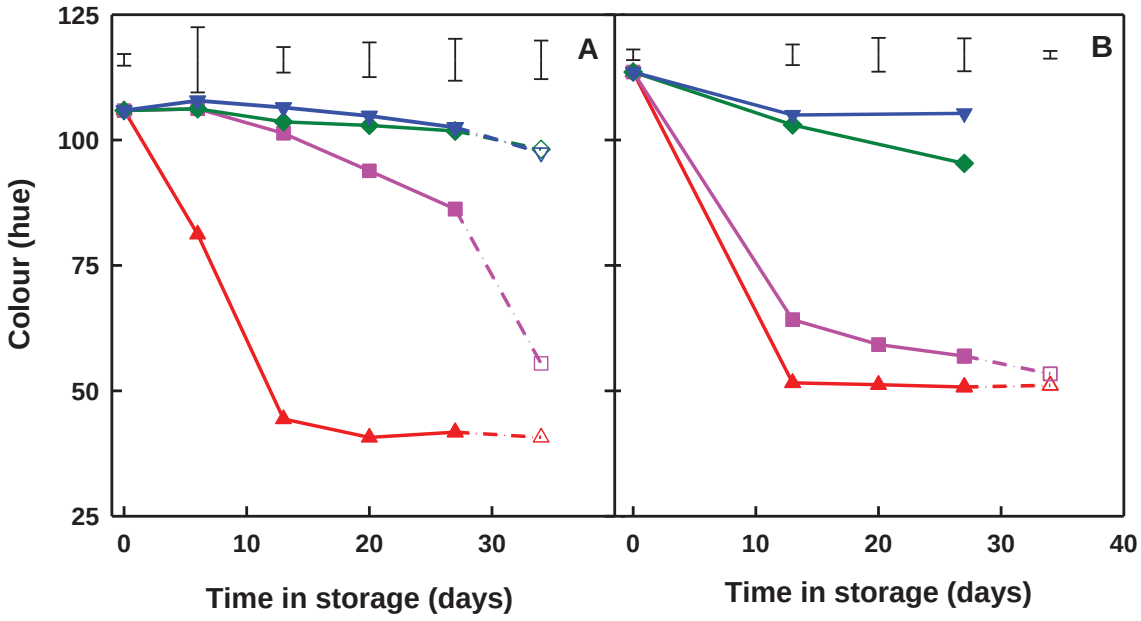

Figure 2.1 Colour development of mature-green tomatoes grown in New Zealand (A) and Florida (B) and stored at 20 (▲), 8 (for New Zealand only) or 12.5 (for Florida only) (■), 6 (♦) or 2.5 °C (▼). Dashed lines with open symbols indicate post-storage removal to 20 °C. Florida-grown tomatoes stored at 2.5 or 6 °C were infected with rot after 27 d storage, resulting in no data being recorded after 27 d. Vertical bars represent LSD α = 0.05.

Tomatoes ('Soraya') grown in Florida and stored for 27 d showed a significant difference in red colour development between chilled (2.5 or 6 °C) and non-chilled (12.5 or 20 °C) fruit ($p < 0.05$). As indicated by reduced hue, the fruit stored at either 12.5 or 20 °C developed red colour, although red colour development for 12.5 °C stored tomatoes was slower than for 20 °C-stored fruit. At 20 °C, hue angle decreased sharply from 113 to 50° after 12 d and stayed almost constant for the remainder of the storage period (Figure 2.1B).

'Soraya' tomatoes stored at 2.5 or 6 °C for 27 d did not develop red colour before removal to 20 °C (Figure 2.1B) and were either pale-yellowish or green in colour after transfer to 20 °C (Figure 2.6). It is possible that chilling exposure at 2.5 or 6 °C caused the fruit to lose the capacity to turn red. Previously, Hobson (1987) reported that at a severe low temperature like 2 °C, one of the most obvious changes in mature-green tomatoes is complete failure to ripen. Many studies found that even breaker tomatoes (which are considered to be less chilling sensitive) failed to attain full red colour when fruit were stored at 3 to 6 °C for 2 to 4 weeks (Gómez et al., 2009; Rugkong et al., 2011).

Overall, results suggest that depending on storage temperature red colour development in tomato can be affected differentially. Storage at 8 °C delayed (but did not perturb) red colour development, fruit maintained at 6 °C showed uneven blotchy red colouration and those at 2.5 °C showed a complete failure of red colouration.

2.3.2. Ethylene production

Tomato is classified as a typical climacteric fruit with a sharp increase in ethylene synthesis at the onset of ripening. 'Cedrico' tomatoes grown in New Zealand held at 20 °C exhibited a maximum ethylene production of 0.04 $nmol.kg^{-1}s^{-1}$ after 12 d before declining (Figure 2.2A). Fruit stored at either 2.5 or 6 °C did not produce any significant amount of ethylene while fruit stored at 8 °C showed a significant increase in ethylene production during 27 d of storage ($p < 0.05$). When all fruit were subsequently transferred to 20 °C after 27 d at low temperatures, ethylene production increased further for fruit stored at 8 °C. A surge of ethylene production occurred for fruit chilled at 2.5 or 6 °C due to post-chilling transfer to 20 °C (Figure 2.2A).

Tomatoes ('Soraya') from Florida stored at 20 or 12.5 ºC increased ethylene production significantly and both showed maximum production around 0.0069 nmol.kg^{-1}s^{-1} after 13 and 20 d of storage respectively. Tomatoes chilled at 2.5 °C had no increase in ethylene production during storage, whereas fruit stored at 6 °C exhibited a slight increase in ethylene production (0.0025 nmol.kg^{-1}s^{-1}) after 27 d (Figure 2.2B).

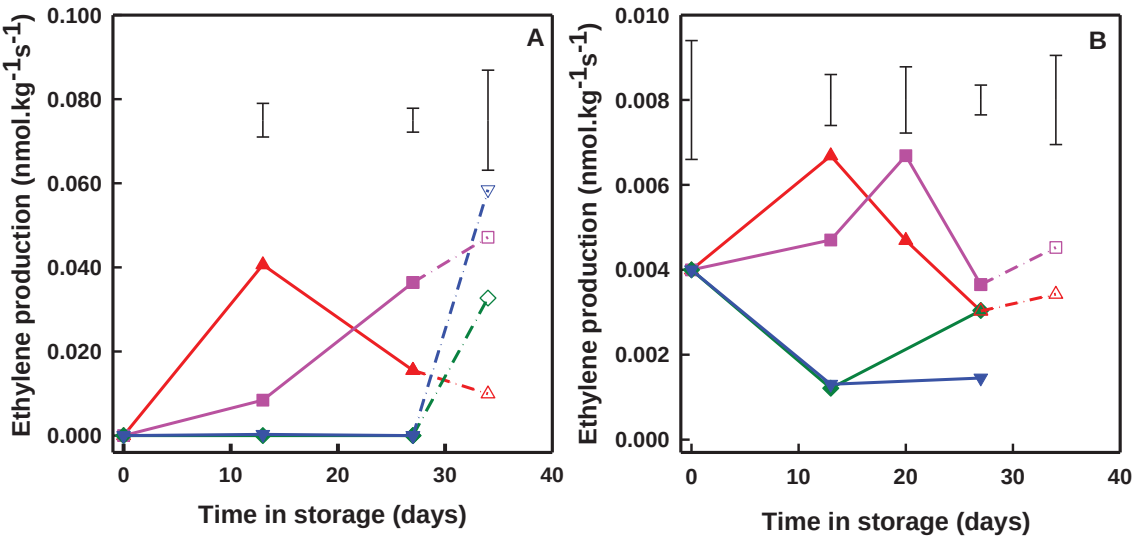

Figure 2.2 Ethylene production of mature-green tomatoes grown in New Zealand (A) and Florida (B) and stored at 20 (▲), 8 (for New Zealand only) or 12.5 (for Florida only) (■), 6 (♦) or 2.5 °C (▼). Dashed lines with open symbols indicate post-storage removal to 20 °C. Florida-grown tomatoes stored at 2.5 or 6 °C were infected with rot after 27 d storage, resulting in no data being recorded after 27 d. Vertical bars represent LSD α = 0.05 at each time of measurement.

The biosynthetic pathway of ethylene production is influenced by ripening, senescence, storage conditions, and biotic and abiotic stresses (Watkins and Ekman, 2004; Lin et al., 2009). When ethylene production data were plotted against the measured hue (colour) (assuming this was an indicator of fruit maturity), it gave a different insight into the relationship of ethylene production and storage temperature. Peak ethylene production of 'Cedrico' tomatoes at 20 °C was seen at a hue angle around 55° (Figure 2.3A). Likewise, at 8 °C peak ethylene was associated with marked colour change. Previously, it was demonstrated that independent of storage temperature, 'Cedrico' tomatoes at the turning stage (hue = 65°) produced the highest amount of ethylene (Biswas et al., 2010), indicating

climacteric peak (Lurie and Klein, 1992). This result suggests that fruit stored at 20 °C exhibited a typical climacteric peak and fruit at 8 °C were likely near to their peak indicated by hue (55°) at the end of post-storage ripening. On the other hand, peak in fruit stored at 2.5 or 6 °C was seen without colour change and only seen during post-chilling warming period i.e. chilled fruit showed a different "trajectory" as compared to non-chilled fruit. During low temperature storage, attribution of observed ethylene production to ripening or stress is difficult (Watkins, 2002). A transient surge in ethylene production is often an indicator of chilling stress particularly when fruit are moved to a non-chilling temperature (Cheng and Shewfelt, 1988). The surge in ethylene production for 'Cedrico' tomatoes stored at 2.5 or 6 °C without significant colour change suggests chilling-stress as the cause. Fruit stored at 2.5 °C also developed decay (Table 2.2 and 2.3) and that decay could further compound increased ethylene production (Barkai-Golan, 2001).

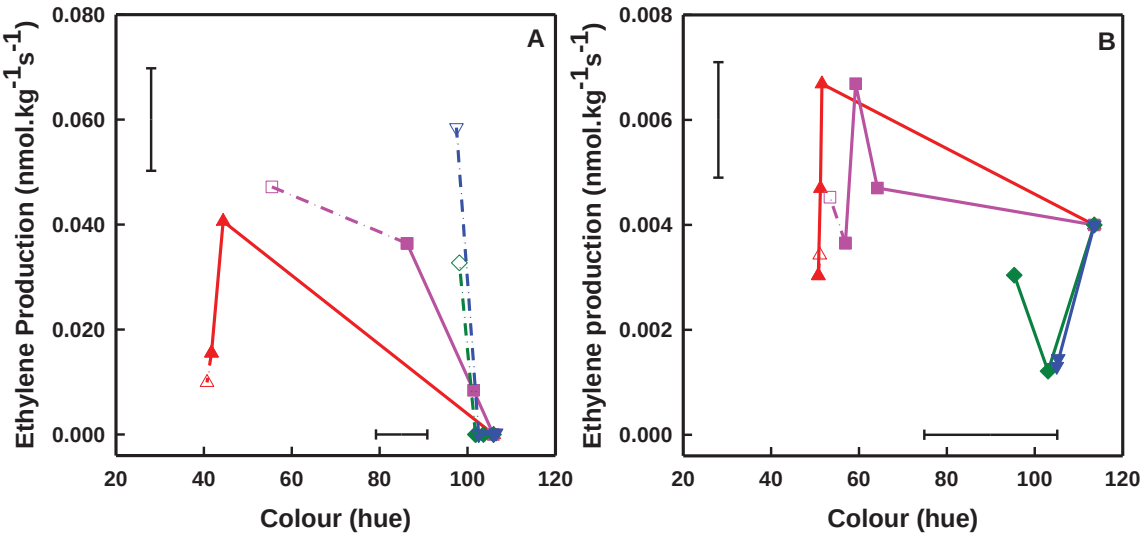

Figure 2.3 Ethylene production of mature-green tomatoes with different hue values. Tomatoes grown in New Zealand (A) and Florida (B) were stored at 20 (▲), 8 (for New Zealand only) or 12.5 (for Florida only) (■), 6 (♦) or 2.5 °C (▼). Dashed lines with open symbols indicate post-storage removal to 20 °C. Florida-grown tomatoes stored at 2.5 or 6 °C were infected with rot after 27 d storage, resulting in no data being recorded after 27 d. Bars represent LSD α = 0.05.

'Soraya' tomatoes stored at 12.5 or 20 °C exhibited highest ethylene production coinciding with a hue angle between 55-60° (Figure 2.3B). This indicates that 'Soraya' tomatoes

ripened at 20 ºC also showed the typical climacteric peak. Fruit stored at 12.5 °C also reached their ethylene climacteric peak although the peak was delayed in comparison to fruit at 20 °C (Figure 2.2B).

2.3.3. Fruit firmness

Fruit firmness was measured only for the tomatoes grown in New Zealand. Firmness (measured by acoustic or compression force) decreased progressively with time during storage and the post-storage period at 20 °C in all treatments. Tomatoes stored continuously at 20 °C showed an exponential decrease in firmness (Figure 2.4). No significant difference in firmness loss was observed between fruit stored at three different chilling temperatures (2.5, 6 and 8 °C) either at the end of storage or end of post-chilling period at 20 °C ($p > 0.05$); however, those fruit softened less than fruit at 20 °C.

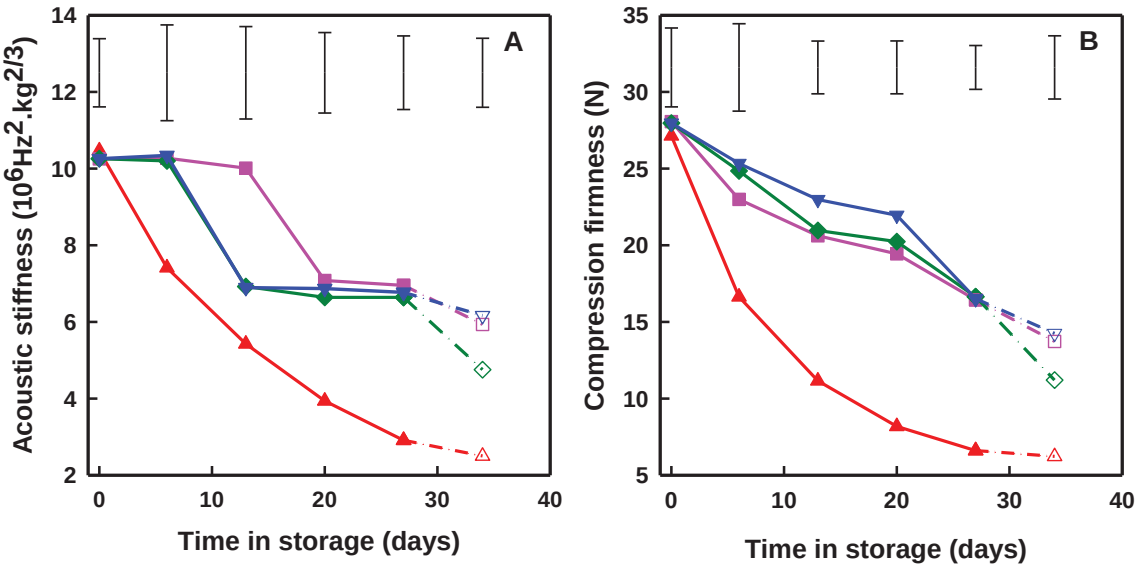

Figure 2.4 Firmness of New Zealand-grown mature-green tomatoes as measured by acoustic stiffness (A) and compression force (B). Fruit were stored at 20 (▲), 8 (■), 6 (♦) or 2.5 °C (▼). Dashed lines with open symbols indicate transfer of fruit from chilling conditions to 20 °C. Vertical bars represent LSD at α = 0.05 at each time of measurement.

Firmness and colour are the most important physical quality parameters in tomato (Tijskens and Evelo, 1994). When acoustic stiffness data were plotted against colour

(assumed as an indicator of ripening), a change in the co-ordination between the two ripening processes was observed (Figure 2.5). During the post-storage period, the loss of stiffness of 20 °C-stored fruit could be attributed to ripening as all fruit turned red. Fruit stored at 8 °C lost stiffness with simultaneous colour change but at a slower rate compared with fruit ripened at 20 °C (Figure 2.5A). However, those 8 °C-stored fruit tracked the fruit stored at 20 °C, suggesting that fruit stored at 8 °C was simply delayed in ripening. It appears that storage at 2.5 or 6 °C decoupled stiffness loss from colour changes whereas storage at 8 °C restored the connection during post-chilling period at 20 °C (Figure 2.5A). Compression analysis also indicated the same trend (Figure 2.5B). It is possible that loss of stiffness without colour change in fruit stored at 2.5 or 6 °C was a chilling-induced effect. Excessive softening in mature-green tomatoes after 2 to 3 weeks of storage at 5 or 7 °C has been previously reported (Efiuvwevwere and Thorne, 1988; Marangoni et al., 1995). Since acoustic response and compression force are largely a measure of the mechanical stiffness of the tissue which is based on both the cell wall mechanical strength and the tension under which the tissue is held by turgor (Hertog et al., 2004), it is possible that softening in fruit stored at 2.5 or 6 °C was primarily due to a loss in turgor.

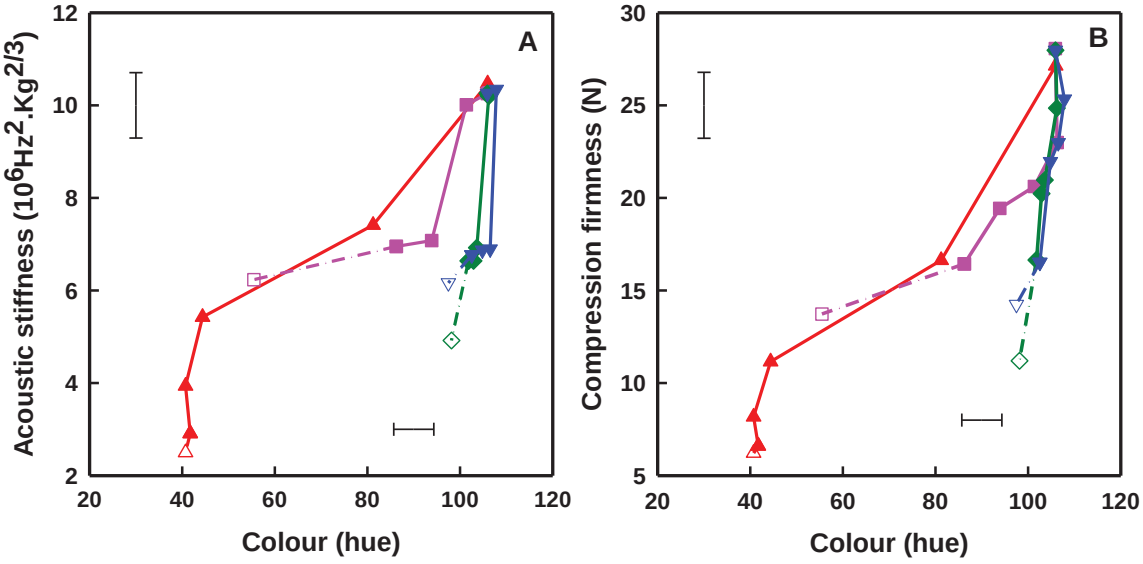

Figure 2.5 Relationship between colour and acoustic stiffness (A) or compression force (B) of mature-green tomatoes stored at 20 (▲), 8 (■), 6 (♦) or 2.5 °C (▼). Dashed lines with open symbols indicate transfer of fruit from chilling conditions to 20 °C. Bars represent LSD at α = 0.05.

2.3.4. Decay incidence and severity

2.3.4.1. Mature-green tomatoes

Decay incidence was assessed during post-chilling period at 20 °C after 13 or 27 d of cool storage. No decay incidence was observed in New Zealand-grown tomatoes during 13 d storage at 2.5, 6 or 8 °C, even after transfer to 20 °C (Table 2.2). However, after 27 d, fruit stored at 2.5 °C developed more decay than fruit at 6 °C. After transfer of 2.5 and 6 °C stored fruit to 20 °C for 7 d, the trend remained the same – the lower the storage temperature, the greater the severity of decay - with all fruit previously stored at 2.5 °C showing infection (100% incidence) (Table 2.2). Decay severity between fruit previously stored at 6 and 8 °C was not different ($p > 0.05$). In this study, isolation of the causal organism was not conducted; however, the black sunken rotten areas on the stem-scar are a characteristic symptom of *Alternaria* infection. Various researchers identified *Alternaria* as the main organism responsible for chilling-induced tomato decay which often develops on the stem-scar of tomatoes (Efiuvwevwere and Thorne, 1988; Artés and Escriche, 1994).

Table 2.2 Decay incidence and severity of New Zealand-grown mature-green tomatoes ('Cedrico') stored under different temperature treatments and transferred to 20 °C for 7 d after 13 d (13 + 7 d 20 °C) or 27 d (27 + 7 d 20 °C) of cool storage.

	13 + 7 d 20 °C		27 + 7 d 20 °C	
	Incd. (%)	Sevr. (0 - 4 scale)	Incd. (%)	Sevr. (0 - 4 scale)
20 °C	0	0	18	0.18 b
8 °C	0	0	9	0.09 b
6 °C	0	0	55	0.55 b
2.5 °C	0	0	100	1.73 a
LSD ($\alpha = 0.05$)		-		0.63

Values in columns followed by a different letter are significantly different at $\alpha = 0.05$.

Florida-grown tomatoes kept at 2.5 or 6 °C for 13 d followed a similar trend to New Zealand-grown tomatoes. However, Florida-grown tomatoes showed higher decay susceptibility during post-chilling removal to 20 °C after 13 d of chilling (Table 2.3). Decay incidence and severity both increased further after 27 d. Negligible decay was found in tomatoes stored at 12.5 or 20 °C, indicating decay observed in fruit stored at 2.5 or 6 °C was chilling-induced (Table 2.3).

Table 2.3 Decay incidence and severity of Florida-grown mature-green tomatoes ('Soraya') stored under different temperature treatments and transferred to 20 °C for 7 d after 13 d (13 + 7 d 20 °C) or 27 d (27 + 7 d 20 °C) of cool storage.

	13 + 7 d 20 °C		27 + 7 d 20 °C	
	Incd. (%)	Sevr. (0 - 4 scale)	Incd. (%)	Sevr. (0 - 4 scale)
20 °C	6	0.06 b	6	0.06 c
12.5 °C	6	0.06 b	6	0.06 c
6 °C	67	1.17 a	100	2.94 a
2.5 °C	89	1.61 a	100	3.06 a
LSD (α = 0.05)		0.55		0.43

Values in columns followed by a different letter are significantly different at α = 0.05.

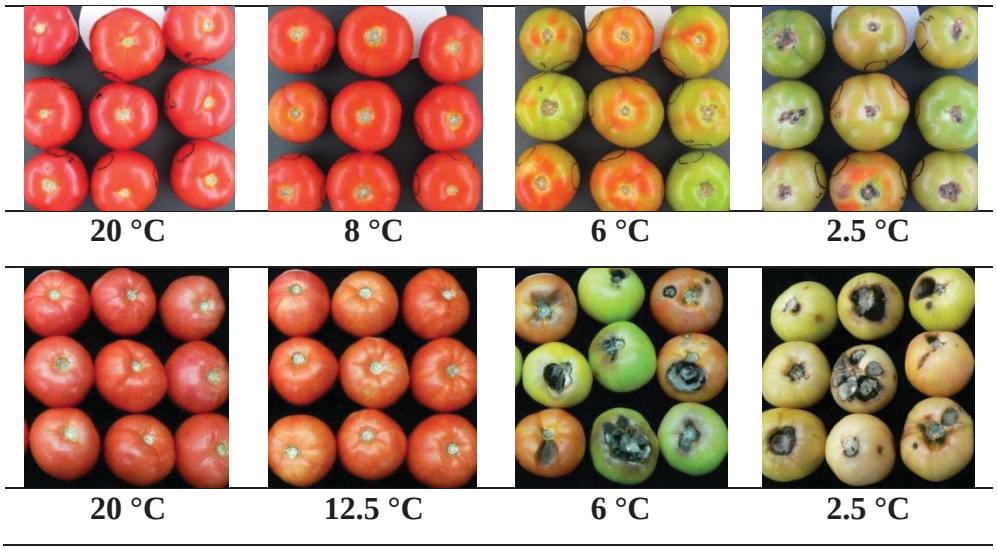

Figure 2.6 Visual appearance of mature-green tomatoes grown in New Zealand (top) and Florida (bottom) and stored under different temperature treatments and transferred to 20 °C for 7 d following 27 d (27+7 d 20 °C) of storage.

Overall, decay severity increased 2 and 6 fold in Florida-grown tomatoes kept in 2.5 and 6 °C respectively in comparison with New Zealand-grown tomatoes following transfer to 20 °C after 27 d of storage. More importantly, unlike tomatoes grown in New Zealand, Florida-grown tomatoes showed decay incidence after 13 d storage at 2.5 or 6 °C. These results suggest that 'Soraya' tomatoes harvested from Florida are more chilling sensitive than 'Cedrico' tomatoes harvested from New Zealand conditions. Pre-harvest growing conditions and/or varietal differences could explain these differences in chilling sensitivity (Bramlage and Meir, 1990; Saltveit, 2005).

2.3.4.2. Harvest maturity influence

Chilling sensitivity in tomato reduces once the fruit are at full ripeness (Autio and Bramlage, 1986). On the other hand, susceptibility to decay (often an indicator of CI) generally increases as tomatoes ripen (Fallik et al., 1993; Prusky, 1996). Although it is possible that mechanisms of ripening associated-decay development differ from chilling-induced decay development, decay symptoms related to ripening and chilling could be confounded during low temperature storage. For instance, Artés and Escriche (1994) identified *Alternaria* in mature-green 'Dario F-150' tomatoes stored for 28 d at 12 °C before removal to 20 °C along with fruit stored at 6 or 9 °C. In their study, fruit stored at 12 °C developed red colour during storage; so decay development for these fruit was more likely ripening associated. Conversely, fruit stored at 6 or 9 °C did not develop red colour, so decay development for those fruit was possibly chilling-induced. In the present study, 'Soraya' tomatoes kept at 12.5 or 20 °C developed full red colour but did not develop decay. On the other hand, all mature-green fruit stored at 6 °C developed decay irrespective of whether some fruit developed red colour (Figure 2.6).

In order to investigate the potential effects of advanced maturity on chilling induced decay incidence, a small experiment using more mature fruit (at harvest) was conducted. 'Soraya' tomatoes were harvested at pink maturity stage and stored at 2.5 or 6 °C for 13 d. During subsequent post-chilling period at 20 °C, fruit from 2.5 and 6 °C both decayed with fruit stored at 2.5 °C having significantly higher decay than fruit at 6 °C ($p < 0.05$) (Figure 2.7); suggesting pink maturity tomatoes were as prone to chilling-induced decay as mature-green fruit. However for both temperatures, pink tomatoes showed lower decay severity than mature-green fruit stored for 13 d (Table 2.3 and Figure 2.7). In summary, the lower the temperature, the higher the decay and decay severity reduces with advancement of ripening.

Although data are limited, it appears that location of decay on the fruit was influenced by fruit maturity. Fruit which remained green during chilling were mostly infected at the stem-scar whereas tomatoes which were able to develop red colour were mainly infected on the surface of the fruit (in particular for 'Soraya tomatoes at 6 °C) (Figure 2.6). This is also true for pink maturity tomatoes where decay largely occurred on the surface of the fruit, not the stem-scar (Figure 2.7). This may be a result of infection of different pathogens at each location. Artés and Escriche (1994) identified *Alternaria* and

Phytophthora in mature-green and *Geotrichum* on breaker tomato stored at 6 or 9 °C for 4 weeks. *Alternaria* is a weak pathogen that usually develops on the stem scar on tomato (Hall, 1965) as resistance to pathogens is easily circumvented due to unavoidable wounding related to harvest. However, it was unclear why ripened fruit or advanced maturity fruit (i.e. pink) showed better resistance to pathogen at stem scar than green fruit. It is possible that with ripening fruit may deposit lignin or suberin in stem scar resulting in the damage due to harvesting being less (Dr D. Huber, personal communication).

6 °C

2.5 °C

	Florida-grown 'Soraya' (pink)	
	13 + 7 d 20 °C	
	Incd. (%)	Sevr. (0 - 4 scale)
6 °C	72	0.91 b
2.5 °C	93	1.51 a
LSD (α = 0.05)		0.58

Figure 2.7 Decay incidence and severity of Florida-grown 'pink' tomatoes ('Soraya') stored under different temperature treatments and transferred to 20 °C for 7 d after 13 d (13 + 7 d 20 °C) of cool storage.

2.3.5. Volatile analysis

Adverse effects on tomato flavour after low temperature storage are documented elsewhere. In the present study, Principal Component Analysis of the flavour profile of tomatoes stored at 12.5 or 20 °C was distinctly separated from tomatoes at 2.5, 6 °C and without any differences between 2.5 and 6 °C-stored fruit (Appendix I). When loading plot was analysed, it was clear that the observed separation between chilled and non-chilled fruit was due to presence of some aroma compounds which are characteristic of ripe-tomato flavour and other aroma compounds which are clustered separately usually represent green tomato (Appendix I). However, the data did not indicate if there was any change in flavour which could be attributed to chilling stress. It simply indicated that lower concentrations of some aroma compounds reported in cool-stored tomatoes (2.5 or 6 °C) were due to effect of reduced ripening under low temperature storage. Detailed explanation is reported in Appendix I.

2.4. Conclusion

Chilling injury in tomato is a physiological disorder with the onset and severity determined by the combination of storage temperature and duration of exposure. Failure to ripen properly, uneven blotchy red colouration and increased susceptibility to decay were found to be the main CI symptoms (Table 2.4) as reported by others (Cheng and Shewfelt, 1988). A strong stimulation of ethylene production during post-chilling period at 20 °C without accompanying colour change perhaps indicated chilling-stress damage.

Table 2.4 Summary of CI symptoms in mature-green tomatoes grown in New Zealand ('Cedrico') or Florida ('Soraya') during storage ≤ 12.5 °C.

Growing location	Storage temp. (°C)	CI symptoms
New Zealand	2.5	Uneven blotchy red colouration Failure to develop full red colour Decay susceptibility Loss in turgor (measured by acoustic or compression force) Increased ethylene production
	6	Uneven blotchy red colouration Loss in turgor (measured by acoustic or compression force) Increased ethylene production
	8	Delayed ripening with no gross CI symptoms
Florida	2.5	Uneven blotchy red colouration Failure to develop full red colour Extreme decay susceptibility
	6	Uneven blotchy red colouration Failure to develop full red colour Decay susceptibility
	12.5	No gross CI symptoms

When New Zealand-grown mature-green tomatoes were stored below 12.5 °C, the nature of CI symptoms was dependent on temperature (Table 2.4). Storage at 8 °C resulted in slower red colour development as compared with storage at 20 °C. However, those 8 °C-

stored fruit ultimately reached a similar ripeness as fruit at 20 °C, indicating delayed (but not perturbed) ripening. No gross CI symptoms were detected in these fruit indicating that 8 °C can be used as a safe storage temperature that would allow New Zealand-grown 'Cedrico' tomatoes to ripen uniformly after storage. Delayed ripening is sometimes considered as a CI symptom (Cheng and Shewfelt, 1988; Sharom et al., 1994). However, as one of the aims of storing fruit at low temperature is to delay ripening, considering delayed ripening as a CI symptom may not be correct. At temperatures below 8 °C increasingly severe damage was caused. When fruit were kept at 6 °C, blotchy red colouration or failure to attain full red colour along with chilling-induced loss in turgor was observed during post-chilling period. Fruit at 2.5 °C failed to develop full red colour, lost turgor and increased decay susceptibility.

Tomatoes grown in Florida and stored under low temperatures (2.5 or 6 °C) also showed uneven red colouration or failure to develop red colour. Firmness was not measured in these fruit and hence turgor changes were unknown. Fruit stored at 6 °C developed decay. Decay susceptibility increased further with decrease in temperature i.e. 2.5 °C. For both storage temperatures, decay was observed after 13 d and severity was doubled after 27 d storage. Fruit harvested even at pink maturity stage was also showed decay at these temperatures, although decay severity of pink fruit was lower than mature-green fruit.

Earlier decay development (13 d for Florida-grown vs 27 d for New Zealand-grown) and higher decay severity for both 2.5 and 6 °C-stored fruit in Florida-grown tomatoes indicates that Florida-grown 'Soraya' tomatoes are more chilling-sensitive than New Zealand-grown 'Cedrico' tomatoes. Pre-harvest growing condition and/or cultivar result in these differences in chilling sensitivity. Decay severity reduced slightly with the advancement of ripening, but both mature-green and pink fruit were susceptible to decay during low temperature storage. This result indicates that harvesting more mature fruit (i.e. pink) to avoid CI and extend shelf life is not a possible strategy as fruit remain susceptible to decay at 2.5 °C.

Results reported in this chapter suggest that storage at chilling temperature affected external fruit colour development independently of the magnitude of chilling temperature (≤ 8 °C). On the other hand, increased decay susceptibility, an important CI symptom during post-storage period, may require severe chilling temperature and a prolonged

exposure (no decay was found for New Zealand-grown tomatoes at 2.5 °C for 13 d). Depending on pre-harvest growing conditions and cultivar, sensitivity of decay susceptibility can be altered as varied results were found between New Zealand and Florida-grown tomatoes. Since initiation of secondary events may appear sequentially, it was possible that different low temperatures would induce different CI symptoms. However, it was not clear whether this was a result of different temperature threshold for damage or just that the phenotype takes longer to display; this question needs to be answered in future research.

3. Increase in electrolyte leakage as a function of chilling stress and ripening of tomato (*)

3.1. Introduction

An increased rate of solute leakage in tissues is often correlated with appearance of CI symptoms and measurement of CI severity in many crops (Saltveit and Morris, 1990). Some crops are exceptions and show no increases in electrolyte leakage as a result of chilling stress. Relative electrolyte leakage (REL) was reported to be unaffected by chilling of peach (Furmanski and Buescher, 1979), bell pepper and eggplant (Murata and Tatsumi, 1979). More importantly, there are some inconsistencies found in terms of electrolyte leakage measurement as an indicator of CI. Bergevin et al. (1993) found a decrease in electrolyte leakage after tomatoes chilled at 1 °C for 14 and 18 d were returned to 20 °C, when CI symptoms appear. Côté et al. (1993) noted that electrolyte leakage decreased drastically when a chilling sensitive tomato variety was returned to 20 °C after 20 and 27 d at 3 °C. Despite such inconsistencies, an increased rate of electrolyte leakage has often been used as an indicator of physical damage of cell membranes in fruit, including tomato (Saltveit, 2005; Antunes and Sfakiotakis, 2008, Zhao et al., 2009b; Dea et al., 2010).

The rate of increase in electrolyte leakage varies with season, crop, and with cultivar. Saltveit (2002) showed an enhancement in electrolyte leakage from tomato ('Castlemart'; harvested in summer) pericarp discs chilled at 2.5 °C after 3 d. However, pericarp discs from the same cultivar harvested during winter exhibited an increase in electrolyte leakage only after 6-7 d of chilling at 2.5 °C (Saltveit, 2005). In cucumber stored at 4 °C, 7-10 d of continuous chilling was required to impart irreversible injury in 'Carolina' and 14 d for 'Marketmore' (Kuo and Parkin, 1989).

Increase in REL as an index of CI has another problem. Apart from species to species variation, seasonal and cultivar differences, there is a serious risk of confounding a chilling-induced increase in REL with the increase in REL accompanying normal fruit ripening. Fruit maturity has a large influence on membrane permeability and solute leakage. Red ripe tomatoes have a higher electrolyte leakage than mature-green tomatoes (King and Ludford, 1983) and a ripening related increase in electrolyte leakage is well documented in many other crops (Lewis and Martin, 1969). Murata (1990) indicated that

(*) Material from this chapter is included in the paper. Biswas, P., East, A. R., Hewett, E. W., & Heyes, J. A. (2012b). Increase in electrolyte leakage as a function of chilling stress and ripening of tomato. *Acta Horticulturae*, *945*, 283-290.

the cellular alterations accompanying fruit ripening may affect membrane permeability and rate of ion leakage. Consequently, it is important to differentiate the increase in electrolyte leakage associated with ripening from the residual change in electrolyte leakage that reflects chilling damage. The main objective of this chapter was to determine if an increase in electrolyte leakage during low temperature exposure could be independently isolated from that caused by postharvest ripening.

Saltveit (2002, 2005) used excised discs of tomato pericarp as a uniform experimental material and keeping discs at low temperature for measuring chilling-induced electrolyte leakage. Pericarp discs are small and easily handled yet they exhibit all the physiological changes found in ripening whole fruit (Saltveit, 1991). A number of other researchers still use whole fruit as experimental material and excise the discs from stored fruit to determine chilling sensitivity (Yang et al., 2009). To verify the validity of this technique this study included a comparison between stored discs and freshly isolated discs from stored fruit to measure electrolyte leakage. Using a disc rather than whole fruit may allow us to reduce fruit to fruit variation.

3.2. Materials and methods

3.2.1. Plant material

Three tomato cultivars (‘Bloody Butcher’ – (BB), ‘Money Maker’ – (MM) and a high lycopene – (HL) cultivar ‘line 3701’) were harvested at the mature-green stage from the Massey University Plant Growth Unit, Palmerston North, New Zealand (for fruit growing conditions refer 2.2.1). For the high lycopene variety, fruit were picked also at pink stage. Fruit were sorted for uniformity and absence of defects and left at room temperature (20 °C) for 24 h before sample preparation.

3.2.2. Sample preparation

Tomatoes were washed with a dilute sodium hypochlorite solution (5% aqueous solution of commercial bleach) and air dried. Pericarp discs were excised with a 12 mm diameter sterile stainless steel cork borer from the equatorial region according to the method described by Saltveit (2005). Discs were rinsed three times with deionised water and gently blotted dry with a tissue paper. Discs were put into plastic petri dishes containing wet

Whatman #1 paper with the epidermis surface down (Figure 3.1). Three to four discs from each fruit were excised and distributed between the treatments to reduce disc to disc variability. Dishes with discs were covered with a lid and put into plastic tubs with a wet paper towel inside the tub. The top was covered with aseptic aluminium foil and petri dishes were placed at 20 °C for 18-20 h to allow dissipation of wound responses (Mencarelli and Saltveit, 1988). All procedures were performed in a laminar flow hood in aseptic conditions.

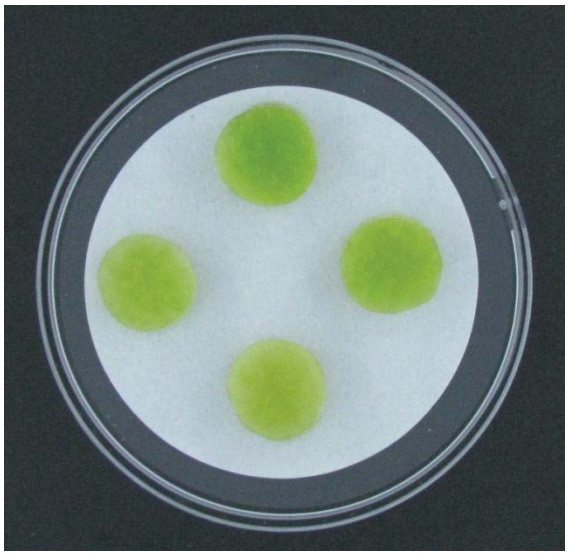

Figure 3.1 Freshly-cut tomato pericarp disc washed 3 times and put into plastic petri dishes containing wet Whatman #1 paper.

3.2.3. Storage conditions

A series of temperatures and times were investigated to determine electrolyte leakage responses. In each experiment ten discs (five replicates with two discs each) from each treatment were used for electrolyte leakage measurement.

In the first experiment, fruit from all three tomato cultivars were tested. The dishes with discs were stored at 6 or 20 °C for 7 d and sampled on d 0, 3, 5, and 7, where day 0 was the day after cutting.

In subsequent studies, only high lycopene cultivar tomatoes were investigated. Discs excised from mature-green fruit were stored at 2.5 °C for 7 d to increase chilling stress and

electrolyte leakage was measured on day 0, 3, 5, and 7. In addition mature-green and pink stage tomatoes were allowed to ripen at 20 °C for 13 d and electrolyte leakage was determined on day 0, 3, 5, 7, 11, and 13.

To determine the long-term chilling effect on electrolyte leakage, discs from mature-green tomatoes were stored at 2.5 or 20 °C for 21 d and electrolyte leakage was measured weekly. Discs stored at 20 °C were monitored for 14 d but no further records were taken due to sample deterioration.

To compare the use of stored discs with freshly excised discs from stored fruit, mature-green fruit were stored at 2.5 or 20 °C for 28 d. Discs were then cut from stored fruit and electrolyte leakage was evaluated weekly. On day 14 and 28, some fruit from 2.5 °C were moved to 20 °C and data were collected after a 6 d period.

3.2.4. Electrolyte leakage measurement

After holding at different temperatures, the dishes with discs were transferred to 20 °C for 1 h before measuring electrolyte leakage. Two pericarp discs were put into 20 mL of an isotonic 0.3 M mannitol solution in a 50 mL centrifuge tube. Isotonic concentration of mannitol for tomato slices was determined using the method described by Saltveit (2002).

Saltveit (2002) reported that for an accurate representation of membrane permeability indicated by an increased rate of ion leakage, the tissue should be bathed in an aqueous isotonic solution. Using pure water as bathing solution may enhance leakage that could simply be the result of osmotic shock imparted from pure water and this would obscure the real permeability (Simon, 1977). Additionally a hypotonic (~ 10 nM) solution may cause additional stress to already damaged tissue (Saltveit, 2002). To determine the isotonic solution, weight gain or loss by the pericarp discs bathed in the mannitol solution was measured by pipetting 20 mL of 0.0 – 0.4 M mannitol solution into each plastic petri dish and gently shaking it on a rotary shaker at 60 cycles min^{-1}. The solution was vacuum-aspirated away from the discs after 20, 60, 120, and 240 min, and the tared dishes weighed and fresh solution added. It was found that at 0.3 M there was no net gain or loss of pericarp disc weight after the initial weight gain and therefore this concentration was considered to be isotonic with the tissue.

Electrolyte leakage (μS/cm) was recorded after 15, 30, 60, 90, 120, and 180 minutes with a conductivity meter (YSI, Japan). Tubes were gently shaken between data recordings. At the end of measurement the tubes were capped, and frozen and thawed once every day over three days. After 3 d the total conductivity was measured at 20 °C after shaking. Electrolyte leakage was determined as the slope of three readings (90 to 180 min – steady linear increases) and expressed as percent of total conductivity (Saltveit, 2002). It was reported that ions in the cell wall and extra cellular spaces within the tissue diffuse rapidly from tissue immersed in an aqueous solution. This results in a transient surge in ion leakage which does not necessarily represent actual chilling-induced permeability of the cellular membrane (Murata, 1990). Therefore, Saltveit (2002) suggested that the rate of ion leakage must be linear during the sampling period. It was found that a transient elevated rate of ion leakage occurred for about 30 min, after which the rate of ion leakage was relatively constant.

3.2.5. Colour

Disc colour was measured with a spectrophotometer (CM-2600D, Konica Minolta Sensing Inc., Japan) on both sides of each disc. Average lightness (L*), chroma (C*) and hue angle (h) of two sides of the disc were calculated.

3.2.6. Statistical analysis

Data was analysed by an ANOVA using SAS software package version 9.2 (SAS Institute Inc., USA) to determine the effect of treatments. A Student's t-test ($\alpha = 0.05$) was performed to test the significant differences in attributes between treatments.

3.3. Results and discussion

3.3.1. Ion leakage measurement on stored disc

When pericarp discs of mature-green tomatoes were stored at 6 or 20 °C for 7 d, discs at 6 °C did not change colour while those at 20 °C developed red colour after 7 d storage as indicated by reduced hue (Figure 3.2 and Figure 3.3). No changes in relative electrolyte leakage (REL) were found in tissues held at 6 °C (Figure 3.2A), whereas discs exhibited an increased REL when stored at 20 °C ($p < 0.05$; Figure 3.2B).

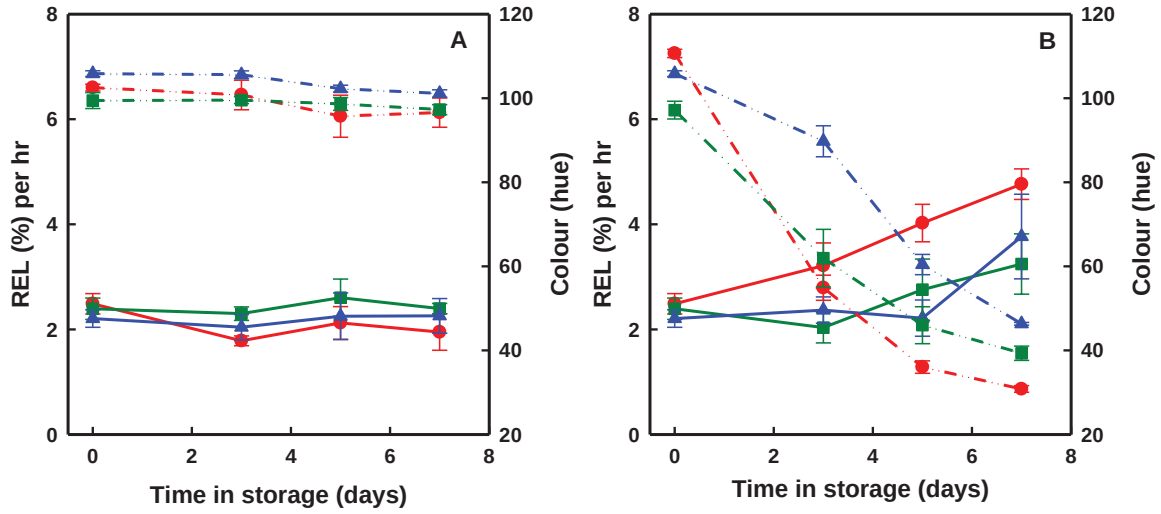

Figure 3.2 Relative electrolyte leakage (solid line) and colour (dashed-dot line) of pericarp discs of mature-green tomatoes (■'Bloody Butcher', ▲'Money Maker', ●'High Lycopene') held at 6 (A) or 20 °C (B). Values represent means ± SE (n=5).

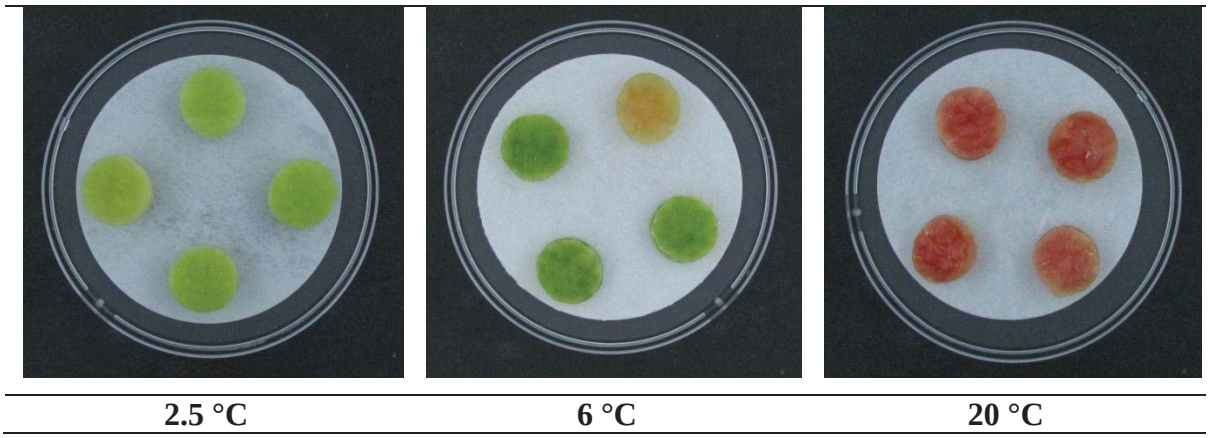

Figure 3.3 Visual quality of pericarp discs of mature-green tomatoes ('High Lycopene') after 7 d at 2.5, 6, or 20 °C.

It is possible that 7 d at 6 °C was insufficient to cause chilling injury since no increase in REL was observed. Previously it was not possible to detect any gross CI symptoms in 'Cedrico' tomatoes stored at 6 °C for 13 d and fruit were able to develop red colour. Chomchalow et al. (2002) did not detect any significant differences in CI as indicated by alteration in final surface colour between tomatoes ('Sunny') stored at 5 °C and non-chilled tomatoes at 12.5 °C stored for 7 d. However, extent of chilling injury damage

indicated as an increased-REL varies with genotype (Kuo and Parklin, 1989) and in this ion leakage study cultivars other than 'Cedrico' were used.

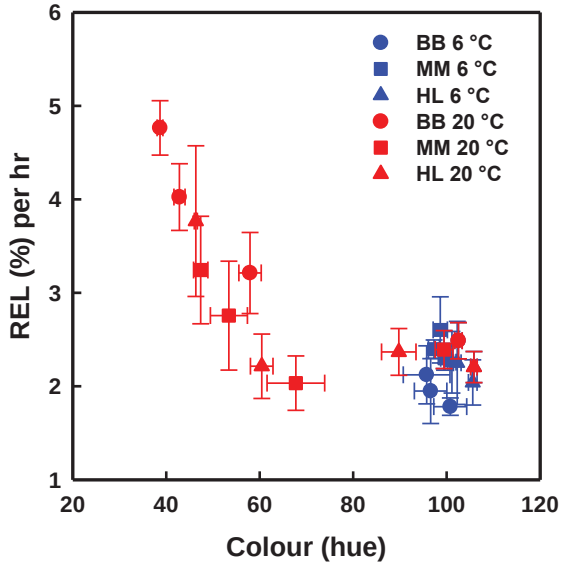

Figure 3.4 Effect of ripeness (colour) of stored pericarp discs for mature-green harvested tomatoes of three cultivars ('Bloody Butcher', 'Money Maker' and 'High Lycopene') on relative electrolyte leakage at 6 or 20 °C. Values are means ± SE (n=5).

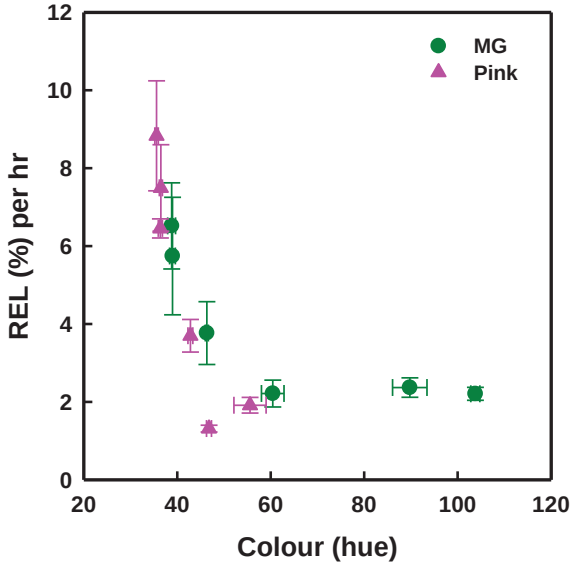

Figure 3.5 Relative electrolyte leakage as a function of ripeness (colour) for stored pericarp discs of tomatoes harvested at mature-green (MG) and pink stages during subsequent ripening at 20 °C for 13 d. Values are means ± SE (n=5).

When REL was plotted against colour, an indicator of ripeness, the increase in REL that occurred during non-chilling temperature may be attributed to ripening (decrease in hue) as discs ripened at 20 °C (Figure 3.4). An increase in membrane permeability, expressed by the change of electrolyte leakage with ripening, is well documented for climacteric fruit including tomato (Lewis and Martin, 1969, Ferguson and Watkins, 1981). When fruit were picked at more mature stages (pink maturity stage), ripening related increases in REL were more dramatic than those harvested less mature (Figure 3.5). These results agree with those of King and Ludford (1983) who reported electrolyte leakage to be around twice as much from red-ripe as from mature-green tomato tissues. It is possible that with ripening, fruit advance in senescence and accelerate in ion leakage (Lyons and Breidenbach, 1987). On

the other hand, it is usually believed that chilling sensitivity reduces as fruit ripen. In tomatoes, immature fruit are more sensitive to chilling temperatures than pink or red tomatoes (Autio and Bramlage, 1986). Therefore, it is logical to suggest chilling sensitivity reduces as fruit ripens when altered colour development is used as a determinant of CI symptom, however, increased ion leakage cannot be used as an unequivocal indicator of CI. It is possible that both ripening and chilling-induced injury may manifest the same physical changes where cells in both senescing and chilled tissue eventually lose their integrity and structure and the consequences of which are measured as increased ion leakage.

When mature-green harvested fruit were stored at 20 °C for 13 d they had reached the red-ripe stage (hue angle of 40°) and REL was approximately 3.8% (Figure 3.5). Conversely, discs from fruit harvested at the pink stage that had developed a similar red-ripe colour after 3 d at 20 °C had an REL of approximately 1.3%. Although data are limited, it is possible that the rate of electrolyte leakage at same colour stage may be increased by ageing (i.e. time after harvest). Electrolyte leakage was increased with fruit senescence and ageing (Knowles and Knowles, 1988). Marangoni et al. (1996) noted that a common feature accompanying senescence was increased membrane permeability, expressed as increased leakage of ions. However, further studies need to be done to understand this relationship and separate out electrolyte leakage as a function of chilling, from that resulting from ageing and ripening. Since both chilling injury and ageing are functions of storage time, it will be a challenge to separate out ion leakage associated with chilling and that occurring as a result of ageing.

When pericarp discs from mature-green tomatoes were stored at 2.5 °C for more than 7 d, there was no increase in REL during first 7 d (Figure 3.6). These results differ from those reported by Saltveit (2002), where REL in discs excised from mature-green tomatoes increased in REL after 3 d at 2.5 °C and doubled after 7 d. In another experiment, Saltveit (2005) found that discs excised from the same tomato cultivar took 6 d before they exhibited a slight increase in REL at 2.5 °C; this was ascribed to seasonal variation. Fruit used in 2002 were grown in high temperatures (>37 °C) and thus were more chilling sensitive than fruit grown during the cooler, fall season (25 °C) in 2005. Tomatoes used in current experiment were grown in a glasshouse during winter (≈ 22 °C), thus may need a longer time to induce CI. This finding also supports our previous hypothesis (see Chapter

2), where it was suggested that tomatoes grown in New Zealand (16-25 °C) were likely less chilling sensitive compared to tomatoes grown in relatively hot Florida climate (21-32 °C).

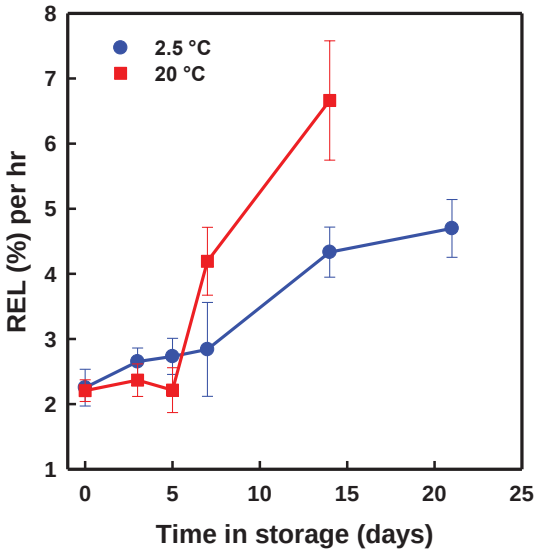

Figure 3.6 Relative electrolyte leakage of pericarp discs stored at 2.5 or 20 °C for 21 d of tomatoes ('High Lycopene') harvested at mature-green stage. Values are means ± SE (n=5). Discs stored at 20 °C had deteriorated after 14 d and no further data were recorded.

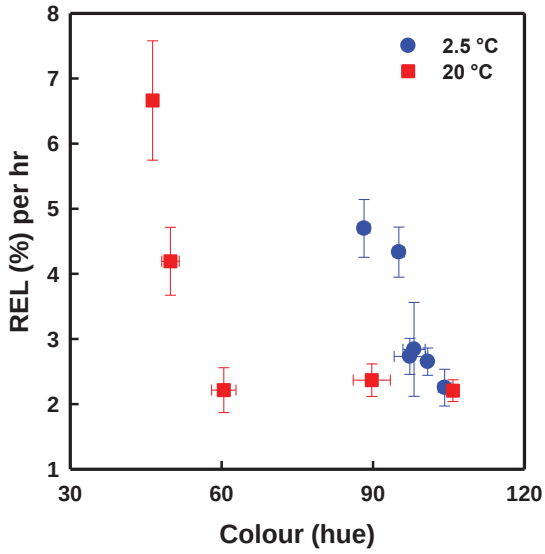

Figure 3.7 Relative electrolyte leakage and colour (hue) changes of pericarp discs stored at 2.5 or 20 °C for 21 d of tomatoes ('High Lycopene') harvested at mature-green stage. Values are means ± SE (n=5).

A significant increase in electrolyte leakage was found after 14 d at 2.5 °C without significant colour change (Figure 3.7). This result indicated that this increase in REL could be independent of ripening (colour change) and therefore chilling-induced. Discs stored at 20 °C showed an expected ripening-related increase in REL in co-ordination with colour development (Figure 3.7).

Overall, an increase in electrolyte leakage is considered an early response to chilling for chilling-sensitive fruit (Saltveit and Morris, 1990), including tomato (Saltveit, 1991),

however, our results indicate that duration of chilling exposure is important. Saltveit (1989) reported that rate of chilling induced ion leakage from tomato pericarp discs increased slowly during chilling and that several days of chilling were required for leakage rates to become significantly greater than for the non-chilled control. More importantly, it was possible to separate chilling-induced increase in REL from ripening-associated changes in REL.

3.3.2. Ion leakage measurement on stored intact fruit

This study also investigated the use of intact fruit and leakage measurement of freshly cut discs from stored fruit, unlike in previous sections where leakage responses were measured using pericarp discs that had been stored. When the discs were excised from mature-green fruit which were stored at 2.5 °C, there was an electrolyte leakage of 1.45% after 7 d, which increased to 2.27% after 14 d (Figure 3.8). After 28 d, REL increased to 3.18% (Figure 3.8). This increase in electrolyte leakage during chilling could not be attributed to ripening, as fruit chilled at 2.5 °C exhibited no significant changes in colour ($p > 0.05$; Figure 3.9).

When fruit were moved to 20 °C after 14 or 28 d at 2.5 °C, REL increased further (Figure 3.8). This was expected as CI symptoms usually appear when fruit are returned to non-chilling temperature and indicates that CI causes increased membrane permeability. Moreover, this increase in REL of fruit stored at 2.5 °C occurred without significant change in colour development i.e. ripening, thus was probably chilling-induced (Figure 3.9). However, a decrease in electrolyte leakage during post-chilling period at 20 °C has been reported (Bergevin et al., 1993, Côté et al., 1993). Fruit stored for 28 d at 20 °C ripened evenly and an expected ripening-related increase in electrolyte leakage was observed over time (Figure 3.9). These results are in accordance with some previous reports where rate of electrolyte leakage in 20 °C stored fruit was significantly higher compared to fruit chilled at 2.5 °C (Lewis and Martin, 1969, Bergevin et al., 1993).

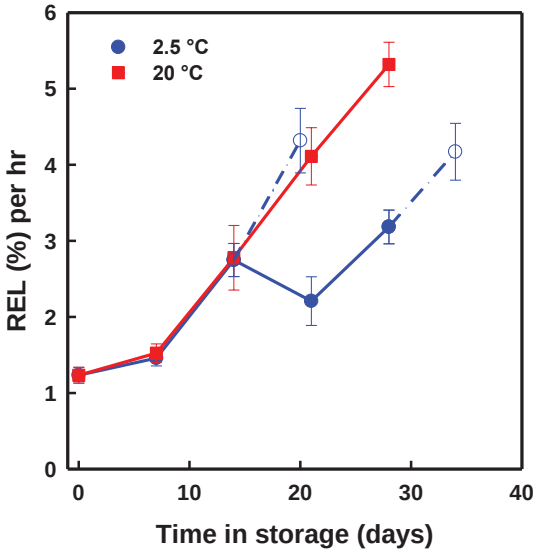

Figure 3.8 Relative electrolyte leakage of freshly excised pericarp discs from tomatoes ('High Lycopene') harvested at mature-green stage and stored at 2.5 or 20 °C. Dashed lines with open symbols indicate post-chilling removal of fruit to 20 °C following 14 or 28 d at 2.5 °C. Values are means ± SE (n=5).

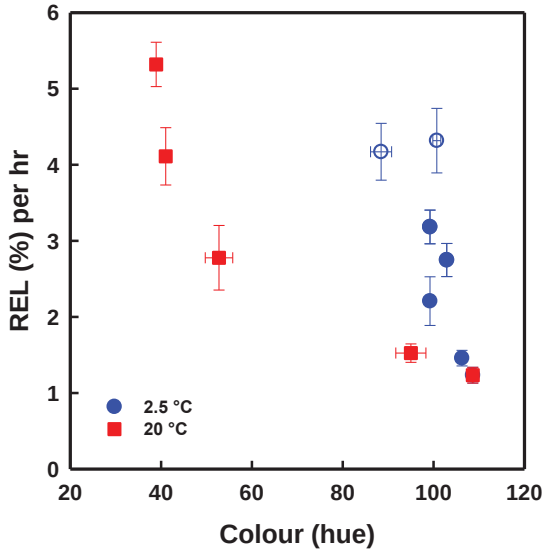

Figure 3.9 Relative electrolyte leakage and colour (hue) changes of freshly excised pericarp discs from mature-green tomatoes ('High Lycopene') stored at 2.5 or 20 °C. Open symbols indicate post-chilling removal of fruit to 20 °C following 14 or 28 d at 2.5 °C. Values are means ± SE (n=5).

Overall, ripening was slower in discs cut from stored fruit than the stored discs and definitely inhibited after 28 d at 2.5 °C. Discs cut from fruit stored at constant 20 °C developed full red colour after 21 d as hue value reduced from 108 to 41°. On the other hand when discs were stored at 20 °C, they developed full red colour after 5-7 d (data not shown), indicating that stored discs developed faster red colour than intact stored fruit. Discs excised from fruit and stored were mechanically more stressed than discs cut from whole fruit which had been stored. It is possible that when discs were excised from fruit and stored, stress received from cutting induced ethylene that accelerated ripening.

In summary, a chilling related increase in relative electrolyte leakage was found both in stored discs (Figure 3.7) and freshly excised discs from stored fruit (Figure 3.9). However

the rate of increase in electrolyte leakage was higher when the discs were stored and stored discs also showed faster ripening compared to intact stored fruit.

3.4. Conclusions

- Pericarp discs of mature-green tomatoes did not show an increase in electrolyte leakage for up to 7 d during exposure to low temperature chilling at 2.5 °C.

- Significant increases in ion leakage were observed in both the stored discs and freshly excised discs from stored fruit at 2.5 °C if stored for longer than 7 d. This increase in electrolyte leakage can be confounded with ripening-associated increase in REL. However, data reported here indicated that it was possible to separate out an increase in REL during low temperature storage from that caused by postharvest ripening by plotting REL against colour. It was found that electrolyte leakage in mature-green tomatoes increased with chilling either in stored discs or in fresh discs cut from stored fruit independently of ripening during longer term (> 7 d) storage.

- Electrolyte leakage of freshly-isolated discs from longer-term stored fruit increased further during a post chilling period at 20 °C. This result suggests that CI symptoms are the result of membrane disturbances during chilling temperature storage, which may not be clearly demonstrated until metabolic rate rises during post-chilling warming.

- Fresh cut discs from stored tomatoes had lower electrolyte leakage than stored discs with same colour score (hue) suggesting that “ageing” of discs could also contribute to the increase in electrolyte leakage. Further research is needed to determine if an increase in electrolyte leakage caused by chilling could be isolated from those caused by “ageing” (time after harvest) process. However, the chilling induced increase in electrolyte leakage is itself a time dependent process.

4. Intermittent warming during low temperature storage reduces tomato chilling injury (*)

4.1. Introduction

Typical CI symptoms in tomatoes have been summarised as uneven blotchy red colouration, excessive softening, and increased susceptibility to decay (Chapter 2; Efiuvwevwere and Thorne, 1988). A transient surge of ethylene production also indicated chilling stress in tomatoes particularly when fruit were moved to a non-chilling temperature (Cheng and Shewfelt, 1988). The nature of CI symptoms can vary depending on storage temperature (Chapter 2).

Interruption of low temperature storage with one or more periods of warm temperature (intermittent warming, IW) was effective in reducing CI and improving market quality in tomatoes (Artés et al., 1998a; 1998b). However, inconsistent results have been reported regarding the effectiveness of IW in reducing CI in tomatoes as IW may require optimization of the rate of temperature change, the extent of temperature change, and the frequency and duration of temperature change. Marcellin and Baccaunaud (1979, as cited in Artés and Escriche, 1994) reported that interruption of cold storage (at 4 or 8 °C for 25 d) with two or three periods of 3 d of warm temperature (20 °C) alleviated CI and decay of breaker stage 'Walter' tomatoes. Artés and Escriche (1994) indicated that 3 cycles of IW to 20 °C for 1 d every 7 d was effective in enhancing surface red colour and reducing CI of 'Darío F-150' tomatoes in Murcia, Spain when fruit were stored at 9 °C, although the same cycles in fruit stored at 6 °C were ineffectual. These results indicate that effectiveness of IW in reducing CI is not consistent.

Since IW can recover the metabolic dysfunction and reverse the manifestation of visible symptoms at 9 °C but not at 6 °C, this suggests that there may be a threshold temperature below which irreversible chilling damage can happen, and IW cannot 'rescue' fruit stored (for too long) below this temperature. Nevertheless, the study of Artés and Escriche (1994) indicated that IW at 6 °C did reduce some CI symptoms such as pitting. It is possible that different CI symptoms have different threshold temperature and responded differentially to IW. Hobson (1981) reported that cyclic warming to 20 °C every 7 d during storage at 2 °C

(*) Material from this chapter is included in the paper: Biswas, P., East, A. R., Brecht, J. K., Hewett, E. W., & Heyes, J. A. (2012a). Intermittent warming during low temperature storage reduces tomato chilling injury. *Postharvest Biology and Technology*, *74*, 71-78.

of mature-green 'Sonatine' tomatoes in the UK invariably led to loss of quality. This indicates that a storage temperature at 2 °C was too low to allow tomatoes to escape CI, even when low temperature period was interrupted with periodic warming to allow metabolic recovery. It is possible that IW is effective only when applied before CI has become irreversible, or IW may be able to reduce CI only in fruit that are stored close to a threshold temperature.

The results of Artés and Escriche (who saw CI after 9 °C storage) differ from our experience with 'Cedrico' tomatoes, which were stored successfully at 8 °C without gross CI development (Chapter 2). It is possible that there are variety-specific temperature thresholds for CI and therefore for the temperatures at which IW may be successful.

The objectives of this chapter were to (i) investigate the effectiveness of an IW regime (based on Artés and Escriche regime) in reducing different CI symptoms of 'Cedrico' tomatoes stored at 2.5 or 6 °C and (ii) apply the same storage temperatures and IW regimes to fruit of a different cultivar from a different production system to investigate differences in response.

4.2. Materials and methods

4.2.1. Plant material and storage conditions

Fruit from each growing location were harvested at mature-green stage. Details of growing and storage conditions were described as in 2.2.1. For New Zealand-grown tomatoes, a total of 45 fruit were randomly grouped into three replicate batches of 15 fruit for each treatment. Next day after harvest, fruit calyces were removed carefully prior to packing into cardboard trays containing a plixtray and a single-layer polyethylene liner. The trays were then placed on wire racks without stacking.

For the Florida experiment, fruit of uniform size and free from defects and blemishes were randomised into 54-fruit lots in three replicates of 18 fruit for each treatment. Six fruit of each replicate were placed in a plastic box on a commercial Styrofoam packaging tray. The trays with tomatoes were placed on a storage rack that was covered with transparent zip-lock plastic to avoid excess air movement.

Fruit were held at 2.5 or 6 °C with 90-95% relative humidity for 27 d. Fruit physiology (ethylene production) and quality (colour, weight loss, firmness, and decay severity) were evaluated during 27 d of storage and a subsequent 7 d post-chilling period at 20 °C. Details of physiology and quality assessment methods used are described in section 2.2. Some fruit were also transferred to 20 °C for 7 d after 13 d of storage with decay incidence recorded. For both locations, three cycles of IW treatments during 27 d storage were achieved by physically shifting fruit from 2.5 or 6 °C to 20 °C after every 6 d of cool storage and returning the fruit to 2.5 or 6 °C after 24 h (i.e. stored at 20 °C on the 7th, 14th and 21st d of storage). Fruit temperatures were recorded by inserting a data logger probe (TGU-1500, Tinytag Ultra Gemini, West Sussex, UK) into the centre of the fruit. It took approximately 4 hr to reach 20 °C of inside fruit temperature from 6 °C and only 1 hr to return to 6 °C from 20 °C. An example of IW (2 cycles) during 6 °C storage is illustrated in Figure 4.1.

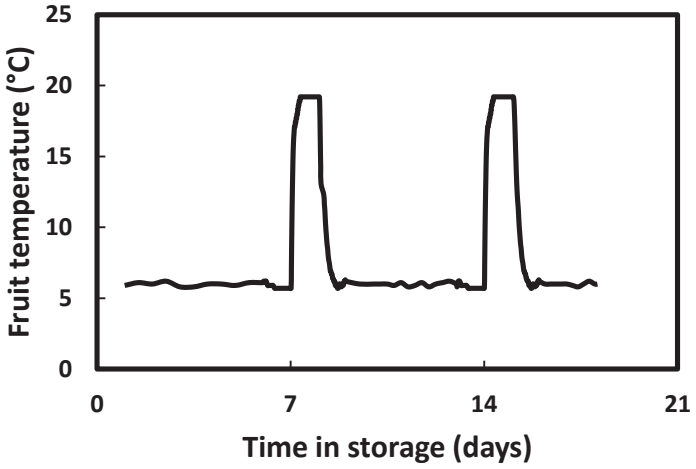

Figure 4.1 Graphical example of an IW regime (6 °C 6 d + 1 d 20 °C).

4.2.2. Statistical analysis

A completely randomized design was used for this study. Analysis of variance (ANOVA) was calculated at each time point using the General Linear Model procedure of SAS (SAS Institute, version 9.2, Cary, NC). The means were compared by the Fisher's LSD test at a significance level of 0.05.

4.3. Results

4.3.1. Surface colour

New Zealand-grown tomatoes stored at 6 °C developed uneven blotchy red colouration whereas 2.5 °C-stored fruit almost lost the capacity to turn red during post-storage period at 20 °C following 27 d storage. IW fruit stored at 6 °C advanced in colour development during storage with fruit turning red when transferred to 20 °C. However, no differences in colour development occurred between IW fruit and continuous chilled fruit at 2.5 °C, even after transfer to 20 °C (Figure 4.2A).

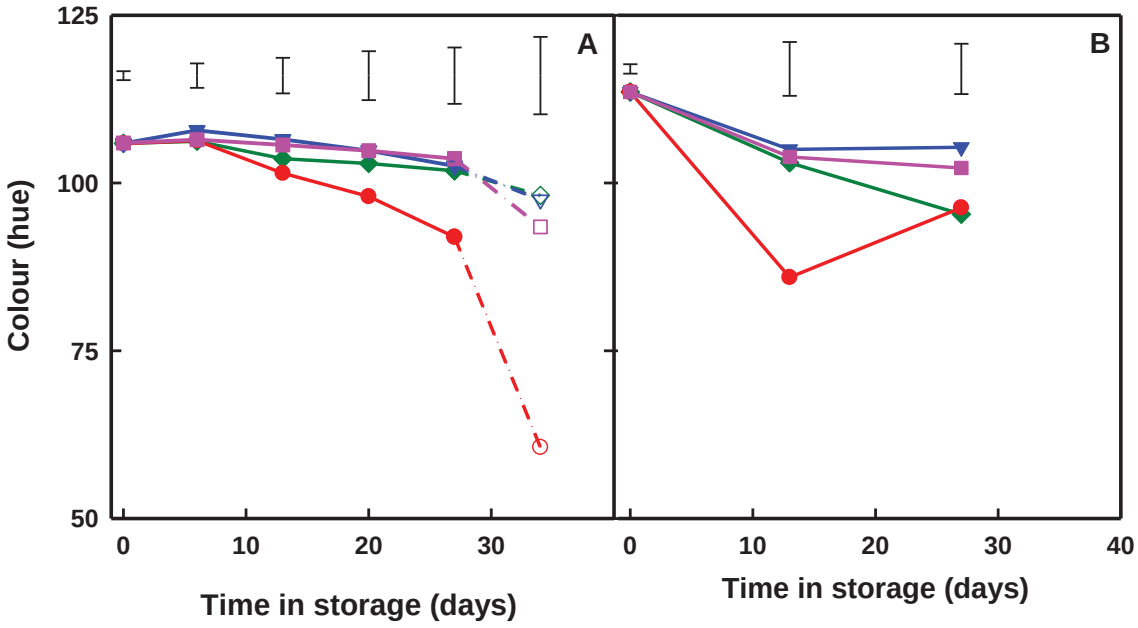

Figure 4.2 Colour development of mature-green tomatoes grown in New Zealand (A) and Florida (B) and stored at 6 (♦), IW at 6 (●), 2.5 (▼) or IW at 2.5 °C (■). Dashed lines with open symbols indicate transfer of fruit from chilling conditions to 20 °C. Florida-grown tomatoes with or without IW were infected with rot after 27 d storage, so no data were recorded after 27 d. Vertical bars represent LSD at α = 0.05 at each time of measurement.

Tomatoes grown in Florida and stored at 2.5 or 6 °C for 27 d did not develop red colour (Figure 4.2B). Importantly, no significant differences in colour development were

observed between chilled and IW fruit during storage at 2.5 or 6 °C ($p > 0.05$) and many fruit in these treatments failed to develop any red colour (Figure 4.2B).

4.3.2. Ethylene production

Tomatoes ('Cedrico') grown in New Zealand and subjected to IW during 6 °C storage produced more ethylene than fruit stored under continuous 6 °C by the end of 27 d storage ($p < 0.05$). However, for IW during 2.5 °C storage, fruit did not produce any detectable ethylene and remained indistinguishable from continuously chilled fruit. When all fruit were subsequently transferred to 20 °C after 27 d at low temperatures, a surge of ethylene production occurred both for fruit continuously chilled and for those subjected to IW (Figure 4.3A). Interestingly, fruit held continuously at 2.5 °C produced significantly higher ethylene than IW fruit from the same temperature upon transfer to 20 °C after 27 d storage. In contrast, IW fruit at 6 °C produced significantly higher ethylene than fruit continuously chilled at 6 °C (Figure 4.3A).

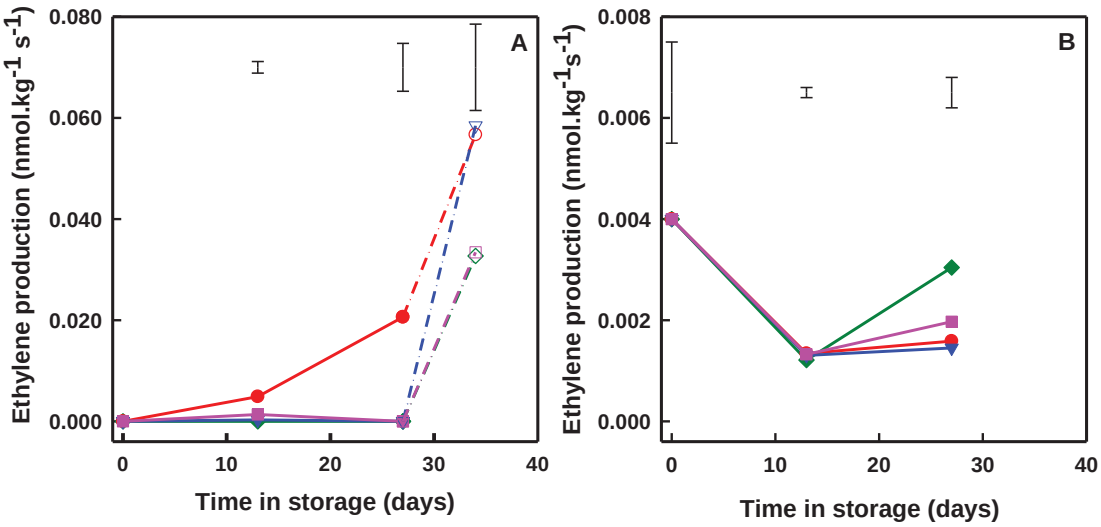

Figure 4.3 Postharvest ethylene production of mature-green tomatoes grown in New Zealand (A) and Florida (B) and stored at 6 (♦), IW at 6 (●), 2.5 (▼) or IW at 2.5 °C (■). Dashed lines with open symbols indicate transfer of fruit from chilling conditions to 20 °C. Florida-grown tomatoes with or without IW were infected with rot after 27-d storage, so no data were recorded after 27 d. Vertical bars represent LSD at $\alpha = 0.05$ at each time of measurement.

Tomatoes ('Soraya') from Florida chilled continuously at 2.5 °C showed no increase in ethylene production during storage, whereas fruit stored at 6 °C exhibited a slight increase in ethylene production (0.0025 nmol.kg^{-1}s^{-1}) after 27 d. Fruit subjected to IW during either 2.5 or 6 °C storage had no increase in ethylene production during the chilling period (Figure 4.3B).

Ethylene production greatly depends on maturation and senescence processes (Autio and Bramlage, 1986). To investigate the ethylene and fruit maturity relationship, data for ethylene production was plotted against colour (hue), which is an indicator of fruit maturity (Figure 4.4). 'Cedrico' Tomatoes subjected to IW at 6 °C storage showed increased ethylene production during storage and developed red colour during post-chilling period at 20 °C (Figure 4.4A). For other treatments, a surge in ethylene production without significant change in colour during post-chilling removal to 20 °C was observed. For 'Soraya' tomatoes from Florida, no clear trend had been observed in ethylene production in relation with colour change (Figure 4.4B).

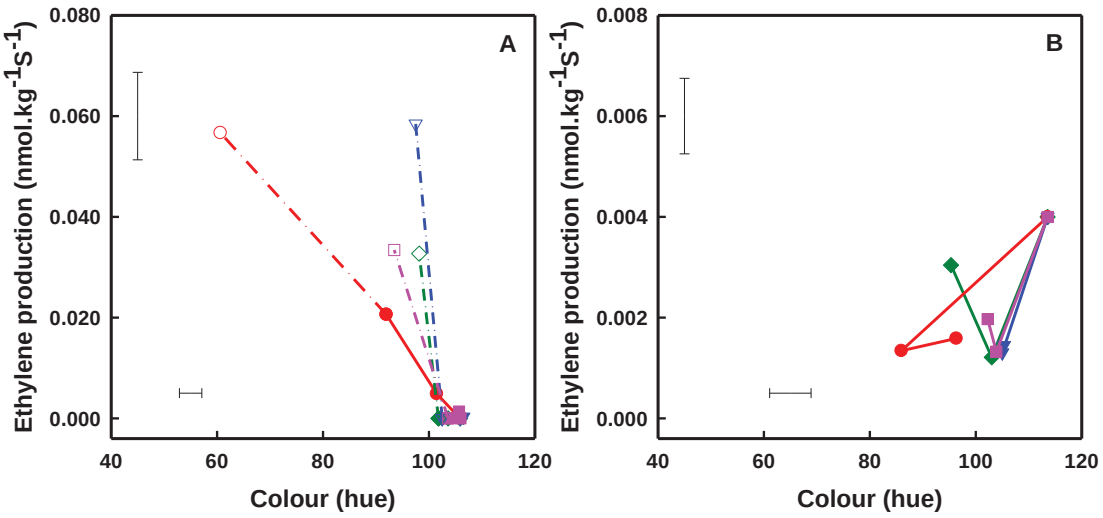

Figure 4.4 Ethylene production of mature-green tomatoes with different hue values. Tomatoes grown in New Zealand (A) and Florida (B) were stored at 6 (♦), IW at 6 (●), 2.5 (▼) or IW at 2.5 °C (■). Dashed lines with open symbols indicate post-storage removal to 20 °C. Florida-grown tomatoes stored at 2.5 or 6 °C were infected with rot after 27-d storage, so no data were recorded after 27 d. Bars represent LSD α = 0.05.

4.3.3. Weight loss

Postharvest weight loss in tomato is usually due to the loss of water through transpiration. In general, weight loss progressively increased with the increasing storage time (Figure 4.5). However, no significant differences in weight loss were found in fruit held under different temperature treatments ($p > 0.05$).

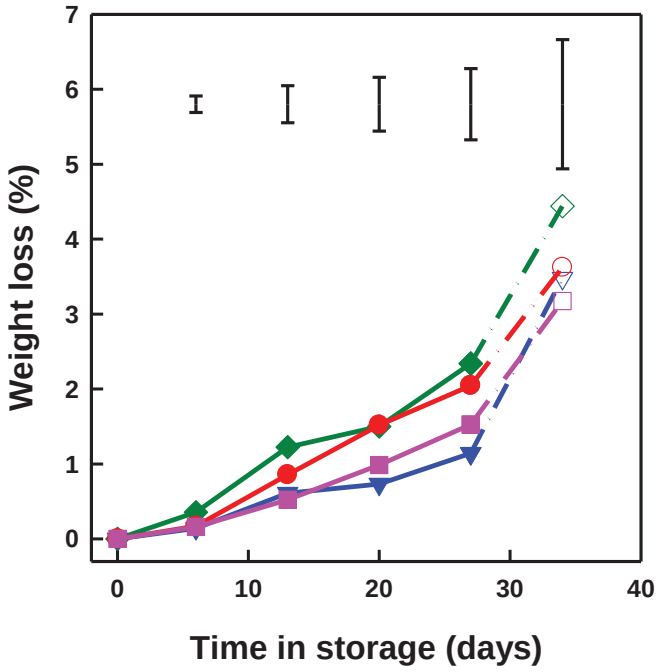

Figure 4.5 Weight loss of New Zealand-grown mature-green tomatoes stored at 6 (♦), IW at 6 (●), 2.5 (▼) or IW at 2.5 °C (■). Dashed lines with open symbols indicate transfer of fruit from chilling conditions to 20 °C. Vertical bars represent LSD at α = 0.05 at each time of measurement.

4.3.4. Fruit firmness

Firmness decreased progressively with time during storage and the post-storage period at 20 °C in all treatments. When firmness was measured by acoustic sensor, a greater but not significant loss in stiffness was observed in fruit that were chilled continuously at either 2.5 or 6 °C than in those subjected to IW ($p > 0.05$) (Figure 4.6A). No significant difference in compression firmness was found between continuously chilled and IW fruit, although IW fruit remained firmer than continuously chilled fruit during the post storage period at 20 °C (Figure 4.6B). No difference in firmness loss was observed between fruit stored at 2.5 and 6 °C.

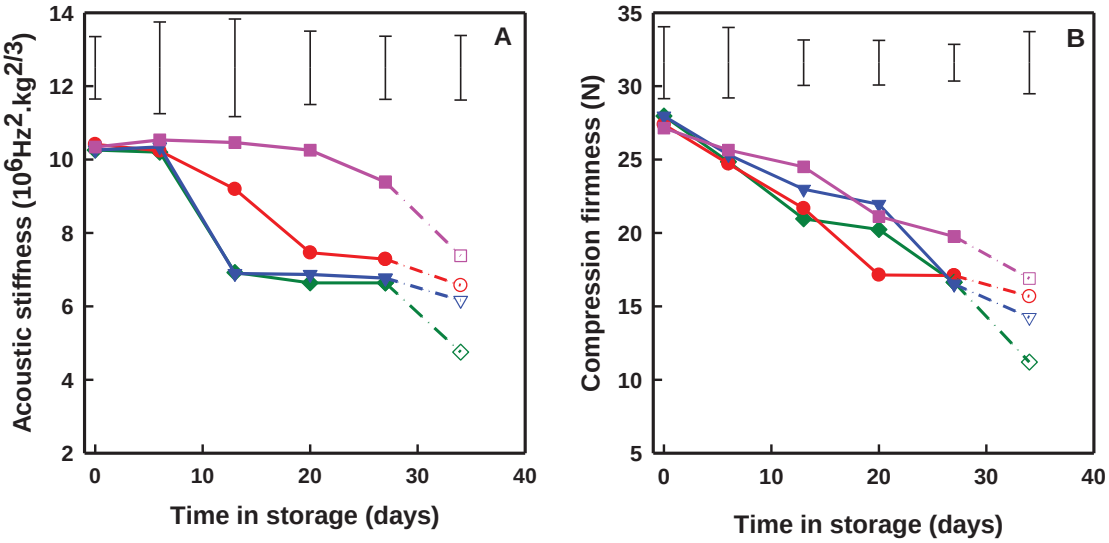

Figure 4.6 Firmness of New Zealand-grown mature-green tomatoes as measured by acoustic stiffness (A) and compression force (B). Fruit were stored at 6 (♦), IW at 6 (●), 2.5 (▼) or IW at 2.5 °C (■). Dashed lines with open symbols indicate transfer of fruit from chilling conditions to 20 °C. Vertical bars represent LSD at α = 0.05 at each time of measurement.

When firmness (acoustic and compression) data were plotted against colour (hue), a change in the co-ordination between the two ripening processes was observed (Figure 4.7). IW fruit at 6 °C developed red colour and decreased stiffness and followed a similar ripening path to fruit stored at 20 °C (refer Figure 2.5). This behaviour is also partially mimicked when tomatoes stored at 2.5 °C were subjected to IW. Most interestingly, IW of fruit stored at 2.5 °C did not stimulate red colour development, but did assist in maintenance of higher stiffness (Figure 4.7A). Compression analysis also indicated the same trend (Figure 4.7B). This result suggests that IW differentially affected firmness and colour change.

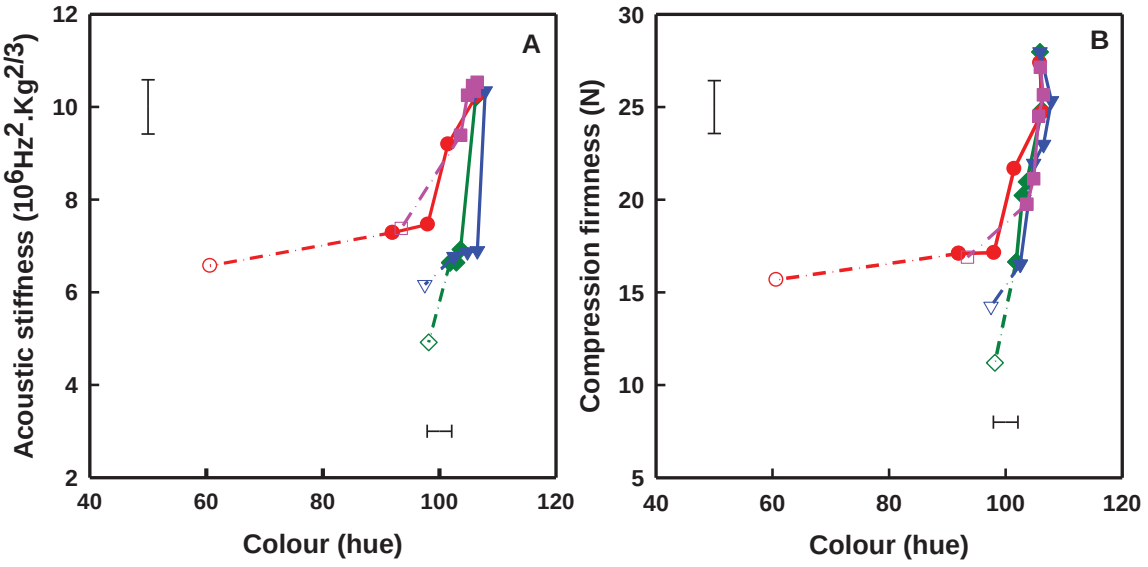

Figure 4.7 Relationship between acoustic stiffness (A) and compression force (B) and colour (hue) of mature-green tomatoes stored at 6 (♦), IW at 6 (●), 2.5 (▼) or IW at 2.5 °C (■). Bars represent LSD at α = 0.05.

4.3.5. Decay incidence and severity

New Zealand-grown tomatoes stored at 2.5 °C for 27 d had significantly higher decay severity than fruit stored at 6 °C ($p < 0.05$, Table 4.1). Three cycles of IW to 20 °C for 24 h every 7 d of storage reduced decay incidence and severity compared with continuous storage at 2.5 or 6 °C ($p < 0.05$, Table 4.1).

Table 4.1 Decay incidence and severity of New Zealand-grown mature-green tomatoes ('Cedrico') stored under different temperature treatments and transferred to 20 °C for 7 d after 13 d (13+7 d 20 °C) or 27 d (27+7 d 20 °C) of cool storage.

	13+7 d 20 °C		27+7 d 20 °C	
	Incd. (%)	Sevr. (0-4 scale)	Incd. (%)	Sevr. (0-4 scale)
6 °C	0	0	55	0.55 b
IW 6 °C	0	0	9	0.09 b
2.5 °C	0	0	100	1.73 a
IW 2.5 °C	0	0	27	0.36 b
LSD (α = 0.05)		-		0.58

Values in columns followed by a different letter are significantly different at α = 0.05.

Table 4.2 Decay incidence and severity of Florida-grown mature-green tomatoes ('Soraya') stored under different temperature treatments and transferred to 20 °C for 7 d after 13 d (13+7 d 20 °C) or 27 d (27+7 d 20 °C) of cool storage.

	13+7 d 20 °C		27+7 d 20 °C	
	Incd. (%)	Sevr. (0-4 scale)	Incd. (%)	Sevr. (0-4 scale)
6 °C	67	1.17 a	100	2.94 a
IW 6 °C	17	0.17 b	67	1.22 b
2.5 °C	89	1.61 a	100	3.06 a
IW 2.5 °C	17	0.17 b	56	0.94 b
LSD (α = 0.05)		0.56		0.64

Values in columns followed by a different letter are significantly different at α = 0.05.

Florida-grown tomatoes kept at 2.5 or 6 °C also showed decay incidence, importantly decay severity increased 2 and 6 fold in Florida-grown tomatoes kept in 2.5 and 6 °C respectively in comparison with New Zealand-grown tomatoes (Table 4.1 and Table 4.2). IW significantly reduced both incidence and severity of decay during 13 d storage ($p < 0.05$); however, effectiveness of IW was reduced after longer term storage (27 d).

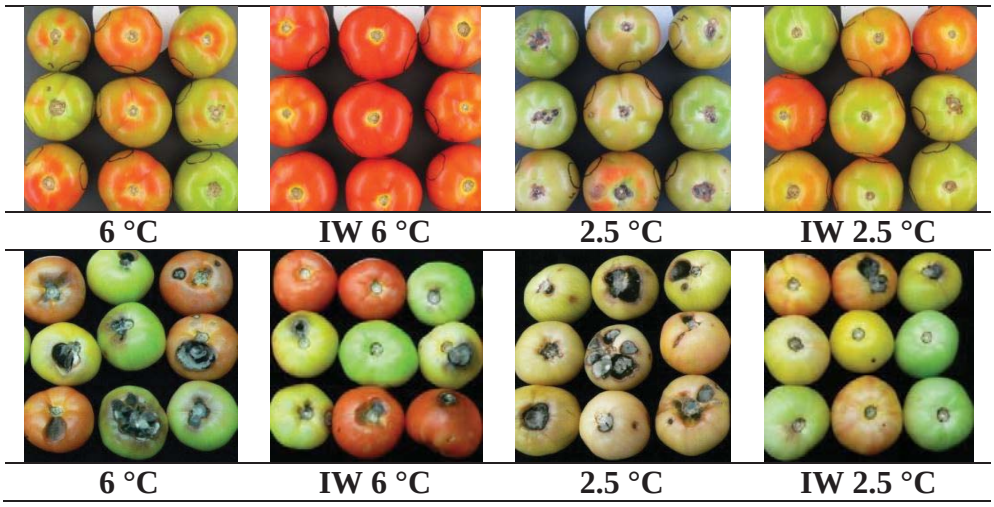

Figure 4.8 Visual appearance of mature-green tomatoes grown in New Zealand (top) and Florida (bottom) and stored under different temperature treatments and transferred to 20 °C for 7 d following 27 d (27+7 d 20 °C) of cool storage.

4.4. Discussion

Chilling injury in tomato is a physiological disorder, the onset and severity of which is determined by the combined effects of storage temperature and duration of exposure to injurious temperature. Failure to ripen properly, uneven blotchy red colouration, chilling-induced excessive softening (particularly measured by acoustic stiffness and compression force) and increased susceptibility to decay were found to be the main CI symptoms. Chilling sensitivity of Florida-grown 'Soraya' was higher than for New Zealand-grown 'Cedrico' (Chapter 2). Pre-harvest growing conditions and/or varietal differences could explain these differences in chilling sensitivity (Saltveit, 2005).

The severity of CI also depends on the duration of exposure to chilling temperature (Saltveit and Morris, 1990). Tomatoes grown in glasshouse in New Zealand took 27 d to show decay at 2.5 °C, whereas outdoor Florida-grown tomatoes showed decay after 13 d at that temperature. These results are reminiscent of previous work investigating the chilling-induced increase in ion leakage in tomato fruit. It was found that tomatoes grown in New Zealand took approximately 14 d to show a significant increase in ion leakage from pericarp discs stored at 2.5 °C versus 3 d reported by Saltveit (2002) for fruit harvested in California during the summer months (Chapter 3, section 3.3.1).

Three cycles of IW to 20 °C for 24 h every 7 d enabled New Zealand-grown tomatoes to develop full red colour subsequent to 27 d of storage at 6 °C. However, the same IW cycles did not allow fruit to turn red after storage at 2.5 °C and fruit remained either green or blotchy coloured. A storage temperature of 2.5 °C was too extreme a chilling injury induction for an effective IW treatment as fruit subsequently failed to develop red colour (Hobson, 1981). Efficacy of IW depends on storage temperature. Artés and Escriche (1994) found that storage of tomatoes at 9 °C with cycles of IW of 1 d at 20 °C every 7 d reduced chilling injury, although the same cycles in fruit stored at 6 °C did not. For Florida-grown 'Soraya' tomatoes, the same IW regime was not effective in enabling red colour development in fruit held at either 2.5 or 6 °C.

New Zealand-grown 'Cedrico' tomatoes stored at 2.5 °C developed decay whereas IW reduced decay significantly. Similarly 'Soraya' tomatoes from Florida also showed decay in fruit stored at 2.5 or 6 °C and IW reduced decay susceptibility in both storage conditions. While IW sometimes was unable to develop full red colour (at 2.5 °C for

'Cedrico' or 2.5 and 6 °C for 'Soraya') but was effective in reducing decay for both cultivars, it is possible that different CI symptoms may have independent responses to IW. The study of Artés and Escriche (1994) who indicated that IW at 6 °C was not effective to alleviate all tomato CI but reduced pitting significantly is also consistent with this idea.

Reduction of decay susceptibility by IW might be related to fruit textural properties. Chilling-induced alteration in texture is well documented in tomato (Jackman et al., 1992; Marangoni et al., 1995; Rugkong et al., 2010). The firmness of both continuously chilled and IW fruit was measured at the same temperature. Loss of fruit firmness without colour change in fruit stored at 2.5 or 6 °C was attributed to chilling. Both the acoustic and compression firmness are influenced by mechanical stiffness and/or turgor of the tissue, with acoustic stiffness known to be more sensitive to water status of the fruit (Hertog et al., 2004). As no significant differences in weight loss were observed between IW and constantly stored fruit at either 2.5 or 6 °C (Figure 4.5), the observed differences in stiffness could relate to cell wall changes or to turgor change (e.g. resulting from damaged cell membranes). Cool storage affects the activities of numerous cell wall modifying enzymes (Brummell et al., 2004), reduces pectin solubilisation and polymerisation (Almeida and Huber, 2008), and promotes loss of turgor (Marangoni et al., 1995). Thus, exposure to CI-inducing temperatures affects cell wall metabolism of tomato fruit (Rugkong et al., 2010), and altering cell wall structure may influence decay susceptibility (Collmer and Keen, 1986). In this study, IW fruit were firmer (stiffer) and had reduced decay compared with fruit held continuously at 2.5 °C. Greater stiffness of IW fruit could be related to maintenance of higher cellular turgor and/or reduced cell wall disassembly. When fruit were transferred to 20 °C after storage, which coincided with decay development, IW fruit still maintained higher stiffness than tomatoes kept continuously at 2.5 °C. It is possible that greater firmness loss at 2.5 °C allowed accelerated decay development by providing greater accessibility to cellular fluid and nutrients that became available for microorganisms. However, chilling induced turgor loss cannot explain positional nature of the rot development we described in section 2.3.4.2. Acoustic or compression firmness is a whole fruit measurement or arguably an equatorial measure yet decay was predominantly localised at the calyx scar. More importantly, tomatoes kept at 6 °C, however, had the same stiffness level as those at 2.5 °C, but they showed significantly less decay, suggesting that cell wall disassembly or turgor alone may not fully explain the increased pathogen susceptibility in chilled tomato (Cantu et al., 2008). It is more likely

that severe chilling injury reduced the ability of the fruit to withstand infection (i.e. reduced disease resistance).

Intermittent warming has been observed to stimulate ethylene production in tomatoes during the warming periods (Biswas et al., 2010). When New Zealand-grown 'Cedrico' tomatoes were stored at 6 °C and subjected to IW, ethylene production was stimulated over time and this response coincided with enhanced colour development. However, fruit that were continuously chilled at 6 °C did not produce comparable ethylene concentrations at cool storage and exhibited uneven red colouration upon post-chilling removal to 20 °C (Figure 4.3). These results suggest that stimulated ethylene during IW may contribute to enabling full red colour development after low temperature storage and the induction of chilling tolerance by advancing maturity. However, it remains to be determined in future whether advanced ripening by IW was a cause or consequence of stimulated ethylene production. 'Cedrico' tomatoes stored at 2.5 °C exhibited a transient surge in ethylene production upon transfer to 20 °C with or without IW, but failed to develop normal red colour. Although it is difficult to separate ripening and stress-related ethylene production (Watkins, 2002), the increase in ethylene production by tomatoes held at 2.5 °C may well be a stress response rather than being associated with ripening (no fruit developed red colour). The lower ethylene production by IW fruit that was observed during the post-chilling period at 20 °C could be related to the less-stressed nature of the fruit. Alternatively, the higher ethylene production for continuous 2.5 °C fruit versus 2.5 °C IW could be a combined function of stress and decay as fruit at that stage also developed rot. Ethylene induction by infected fruit tissue is well reported (Barkai-Golan and Kopeliovitch, 1983). The same trend of ethylene production was also found for Florida-grown 'Soraya' tomatoes on day 27 (Figure 4.3). A slight but not significant increase in ethylene production for IW fruit at 2.5 °C and significant increase in 6 °C-stored fruit could be associated with decay incidence.

Links between ethylene and decay incidence have been previously proposed. Exogenous ethylene has been observed to stimulate decay in tomato (Segall et al., 1974), whereas other studies have shown that exposure of tomato to ethylene may lead to induced resistance to postharvest pathogens (Geeson et al., 1986). Diáz et al. (2002) suggested that ethylene perception is required for increased resistance of tomato leaves to *Botrytis cinerea*; however, the situation for fruit is complicated by the increased decay

susceptibility that occurs during ripening. Su and Gubler (2012) demonstrated that 1-MCP, which inhibits ethylene perception, reduced postharvest decay in mature-green tomatoes at 18 °C, but Baldwin et al. (2011) found that 1-MCP treatment resulted in increased decay in partially ripe tomatoes. In the former case, 1-MCP kept the fruit in the more resistant unripe condition, while the later 1-MCP may have interfered with ethylene-induced resistance in ripening tomato fruit. New Zealand-grown tomatoes subjected to IW during 2.5 °C storage did not produce any significant quantity of ethylene during 4 weeks of storage yet decay was significantly reduced. For Florida-grown tomatoes, IW during 2.5 and 6 °C storage also reduced decay, but did not result in any significant increase in ethylene production. It seems IW reduced the decay incidence and severity significantly for both tomato cultivars grown in different regions with or without ethylene stimulation, suggesting a more direct effect of IW on CI-related decay susceptibility.

4.5. Conclusion

Previously, it was demonstrated that Florida-grown 'Soraya' tomatoes were more chilling-sensitive than New Zealand-grown 'Cedrico' tomatoes (Chapter 2). It was further suggested that when mature-green tomatoes were stored under optimal storage temperature, the nature of CI symptoms can vary depending on the temperature. Results reported in this chapter suggest that –

- IW can enhance red colour development and reduce decay in tomatoes stored at 2.5 or 6 °C, but the magnitude of effects depend on cultivar and growing conditions. More importantly, different CI symptoms in tomatoes have independent responses to IW.

- IW stimulated ethylene production and advanced red colour development in fruit stored at 6 °C. However, it was unclear whether stimulated ethylene production was a cause of or just a correlation with advancing red colour development. Elimination of IW-stimulated ethylene response by use of 1-MCP may provide an answer whether this ethylene has a causal relationship in reducing CI in tomato. This relationship will be explained in detail in chapter 6. Additionally, since chilling sensitivity in tomato usually reduces once the fruit advance to full ripeness and tomatoes treated with 1-MCP usually shows delayed ripening, it is important to determine whether application of 1-MCP

increases chilling sensitivity by delaying ripening during cool storage. Role of 1-MCP in influencing ripening and chilling injury will be discussed in chapter 5.

- IW reduced chilling induced-decay. It was possible that reduction of rot by IW may not be ethylene mediated, although further research is needed to clarify the role of IW-stimulated ethylene production influencing chilling-induced decay.

- Overall, while IW can be effective in reducing CI, it is clear that general recommendations for IW may not be appropriate as the responses are highly dependent on cultivar or production conditions. As both cultivars were not analysed at both locations, the independent role of genotype and production conditions could not be discriminated, but does present itself as a suitable research question for the future.

5. Ripening delay caused by 1-MCP may increase tomato chilling sensitivity

5.1. Introduction

The role of ethylene in inducing ripening of climacteric fruit is well documented (Barry and Giovannoni, 2007). Tomato is a climacteric fruit and its ripening is highly dependent on ethylene action (Alexander and Grierson, 2002). Importantly, ethylene is required even at advanced stages of tomato maturity (when ripening has already started) (Hoeberichts et al., 2002) and probably acts as a regulator for ethylene-dependent processes in ripening (Theologis, 1992). The ethylene effect on harvested products can be beneficial or detrimental depending on the product, its ripening stage, and its desired use (Saltveit, 1999). The rapid ripening of fruit after harvest often limits postharvest life and thus long distance marketing (Saltveit, 1999). Therefore, in industry attempts are made to avoid exposure of many horticultural commodities to ethylene or at least minimise ethylene production and action to aid long term postharvest handling.

A potent inhibitor of ethylene action, 1-MCP has emerged as a tool for controlling ripening and extending shelf life during postharvest handling of a number of fruit and vegetables (Blankenship and Dole, 2003; Watkins, 2006). 1-MCP blocks ethylene receptors so that ethylene cannot bind and elicit action (Sisler and Serek, 1997; Blankenship and Dole, 2003). 1-MCP is nontoxic and has negligible residue (Watkins 2006). Application of 1-MCP prolongs storage life of tomato by lowering ethylene production, respiration rate, loss of firmness and titratable acidity, and inhibiting colour development including lycopene accumulation or chlorophyll degradation (Wills and Ku, 2002; Opiyo and Tie-Jin, 2005; Guillén et al., 2006; Choi and Huber, 2008; Sabir and Agar, 2011). The influence of 1-MCP on postharvest decay during ripening is less clear as 1-MCP can increase (Hurr et al., 2005; Baldwin et al., 2011) or decrease (Guillén et al., 2006; Su and Gubler, 2012) decay incidence in tomato.

Efficacy of 1-MCP in maintaining fruit quality or extending storage life is related to cultivar, 1-MCP concentration, exposure duration, and ripening stage (Mir et al., 2004; Hurr et al., 2005; Guillén et al., 2007). The effect of 1-MCP in delaying or inhibiting ripening was greater in tomatoes harvested at the mature-green stage than pink stage (Sabir

and Agar, 2011). Tomatoes treated with 1-MCP at early ripening stages had a longer storage life than those of advanced ripening stages (Hoeberichts et al., 2002). However, Hurr et al. (2005) suggested that 1-MCP treatment could be of little benefit and possibly detrimental if applied to early maturity fruit (green and breaker), due to fruit failing to soften to acceptable level and develop abnormal colour. Fruit may develop acceptable colour and soften eventually, however, prolonged period of delay in ripening often resulted in compromised fruit quality due largely to pathogen incidence and water loss (Hurr et al., 2005). Treatment with 1-MCP of tomatoes harvested at the advanced ripening stage (pink or ripe) was effective in extending shelf life (Hurr et al., 2005) and maintaining fruit quality (Guillén et al., 2006). It was possible that tomatoes at advanced stage of ripening had already developed organoleptic properties (e.g. aroma development) and thus fruit could reach consumers with better quality (Guillén et al., 2006; Balwdin et al., 2011).

Tomatoes are susceptible to CI when stored below 13 °C for 2 weeks or more. The role of 1-MCP in influencing tomato CI is not well defined. Most studies with 1-MCP show delayed ripening of tomatoes stored at room temperature or above chilling temperatures (Hoeberichts et al., 2002; Mostofi et al., 2003; Mir et al., 2004; Baldwin et al., 2011; Sabir and Agar, 2011; Su and Gubler, 2012). Few studies have been reported on the effect of 1-MCP on tomato CI particularly with mature-green fruit which is more chilling-sensitive than advanced maturity fruit. Jeong et al. (2004) reported that 1-MCP reduced water soaking development and maintained texture quality of fresh-cut tomato slices stored at 5 °C, although the authors did not attribute the water soaking of tomato slices as a CI symptom. Jing and Zi-Sheng (2011) reported that 1-MCP treated (1 $\mu L.L^{-1}$) mature-green tomatoes (‘Zheza 205’) showed delayed ripening and more chilling injury than non-treated fruit stored at 3 °C for 14 d. On the other hand, Tadesse et al. (2012) suggested that 1-MCP (2.9 $\mu L.L^{-1}$) had no effect in delaying colour development or firmness loss in breaker or red tomatoes (‘Roterno’) stored at 4 or 8 °C for 24 d. Perhaps differences in 1-MCP effect in influencing CI were due to differences in fruit maturity, cultivar used or 1-MCP concentration.

Similarly in other fruit, the effect of 1-MCP associated with CI is not consistent. Pre-storage application of 1-MCP to avocados caused a reduction in mesocarp discoloration and decay development (Pesis et al., 2002), but did not reduce chilling-induced external skin blackening (Woolf et al., 2005). Similarly, inhibiting ethylene action by 1-MCP

reduced CI in climacteric plums (Candan et al., 2008), melons (Ben-Amor et al., 1999), persimmons (Salvador et al., 2004) and in non-climacteric fruit such as pineapples (Selvarajah et al., 2001). 1-MCP also reduced chilling-induced superficial scald in apples (Fan et al., 1999) and pears (Argenta et al., 2003). On the other hand, bananas treated with 1-MCP showed increased CI symptoms as indicated by failure to ripen or uneven colour development (Jiang et al., 2004). Equally, inhibition of ethylene action by 1-MCP induced a greater incidence of woolliness in peaches (Zhou et al., 2001) and nectarines (Dong et al., 2001). Since 1-MCP blocks ethylene action primarily and the role of ethylene in manipulating CI was complex, it is no surprise 1-MCP induces varied responses to CI in different crops.

Since chilling sensitivity in tomato usually reduces once the fruit advance to full ripeness and tomatoes treated with 1-MCP showed delayed ripening, it is possible that 1-MCP increases chilling sensitivity by delaying ripening. The objectives of this present chapter were to examine the effectiveness of 1-MCP on 'Cedrico' tomato in delaying ripening, when applied to fruit at varying developmental stages including mature-green, breaker, turner, pink, and ripe. Additionally, in order to determine the effectiveness of 1-MCP on CI of tomato fruit, mature-green and breaker tomatoes were treated with 1-MCP before being stored at 2.5 °C for 35 d.

5.2. Materials and methods

5.2.1. Plant material

Tomatoes ('Cedrico') were grown at Massey University Plant Growth Unit, Palmerston North (for fruit growing condition refer to section 2.2.1). Fruit of uniform size and free from defects were visually sorted by colour using USDA standard colour classification chart (USDA, 1975) and classified as 5 different maturities (mature-green, breaker, turner, pink and ripe). Fruit calyces were removed carefully and fruit were left at room temperature (20 °C) for 24 h before 1-MCP treatment.

5.2.2. 1-MCP treatment

Fruit were divided into two groups and treated with either 1-MCP or air. For 1-MCP treatment, 0.45 g of a 1-MCP-releasing powder (SmartFresh^{TM}, 0.14% a.i., AgroFresh,

Queensland, Australia) was dissolved in 3 mL of milliQ water in a 100 mL syringe as suggested in AgroFresh Inc. guidelines. A final gas concentration of 5 $\mu L.L^{-1}$ of 1-MCP was used to treat 30 tomatoes placed in a 56 L airtight sealed container for 24 h at 20 °C (Figure 5.1). The non-treated control i.e. 1-MCP (-) fruit were also held in an identical sealed container with air for 24 h at 20 °C. After 24 h treatment, the sealed containers were opened outdoors and fruit were placed in cardboard trays containing a plixtray and single-layer polyethylene liner. The trays were placed on wire racks without stacking.

Figure 5.1 Pre-storage application of 1-MCP (5 $\mu L.L^{-1}$) to the fruit placed in a 56 L sealed container. Fruit were held at 20 °C for 24 h before venting the chambers and transferring fruit to cool store.

5.2.3. Storage conditions

Two separate experiments were conducted. In the first experiment, tomatoes of 5 different maturities at harvest were stored at 20 °C for 14 d. A total of 12 fruit for each maturity were treated with 1-MCP. Fruit with (+) and without (-) 1-MCP treatment applied before storage were held at 20 °C in two separate cabinets respectively to avoid cross-contamination. Fruit colour (hue), acoustic stiffness and visual appearance were evaluated after 7 and 14 d of storage.

For the second experiment, only two maturities (mature-green and breaker) were treated with (+) or without (-) 1-MCP. Cardboard boxes containing treated fruit (covered with single layer polyethylene liner) were placed at 2.5 or 20 °C for 35 d. Fruit quality (colour,

stiffness and decay severity) and visual appearance were evaluated at the end of 5 weeks storage and a subsequent 4 d post-chilling period at 20 °C.

For both experiments, colour (reflectance spectrophotometer) and stiffness (acoustic sensor) were measured as previously described in section 2.2.2 and 2.2.4 respectively. Decay severity was assessed using 0 to 4 visual rating scales (Chapter 2, section 2.2.5).

5.2.4. Statistical analysis

Effect of treatments was analysed using the General Linear Model procedure of SAS (SAS Institute, version 9.2, Cary, NC). When appropriate, means of different treatments were compared using Fisher's least significant difference at the 5% level.

5.3. Results and discussion

5.3.1. Effect of 1-MCP on colour development in tomatoes ripened at 20 °C

Tomatoes harvested at five different maturity stages were allowed to ripen at 20 °C for 14 d. At harvest hue angle of mature-green (MG), breaker, turner, pink, and ripe tomatoes was 102°, 93°, 84°, 70°, and 57° respectively (Figure 5.2). Treatment with 5 $\mu L.L^{-1}$ 1-MCP delayed red colour development in all maturities of tomatoes; however, the efficacy of 1-MCP varied significantly with fruit maturity at time of application.

After 7 d of storage, hue angle of 1-MCP treated MG fruit did not change from the value at-harvest with the first signs of colouration occurring 14 d after the start of the experiment (Figure 5.2 and Figure 5.3). 1-MCP treatment significantly delayed further colour development of breaker, turner, pink, and ripe fruit for the first 7 d. In contrast, control fruit of different maturity tomatoes almost all reached a red colour after 7 d as mean hue value of all maturities was around 50° (Figure 5.2A).

After 14 d, treated MG tomatoes had not yet reached the breaker stage (hue = 94°), while non-treated control fruit had obtained the full red colour (hue = 43°) (Figure 5.2B). Red colour development of other maturities (breaker to ripe) was also delayed by 1-MCP as compared to control fruit. Importantly, 1-MCP prevented breaker, turner or pink fruit from

attaining full red colour (< 50°) after 14 d and skin colour of these fruit were orange-yellow, whereas ripe fruit reached full red colour (Figure 5.2 and Figure 5.3).

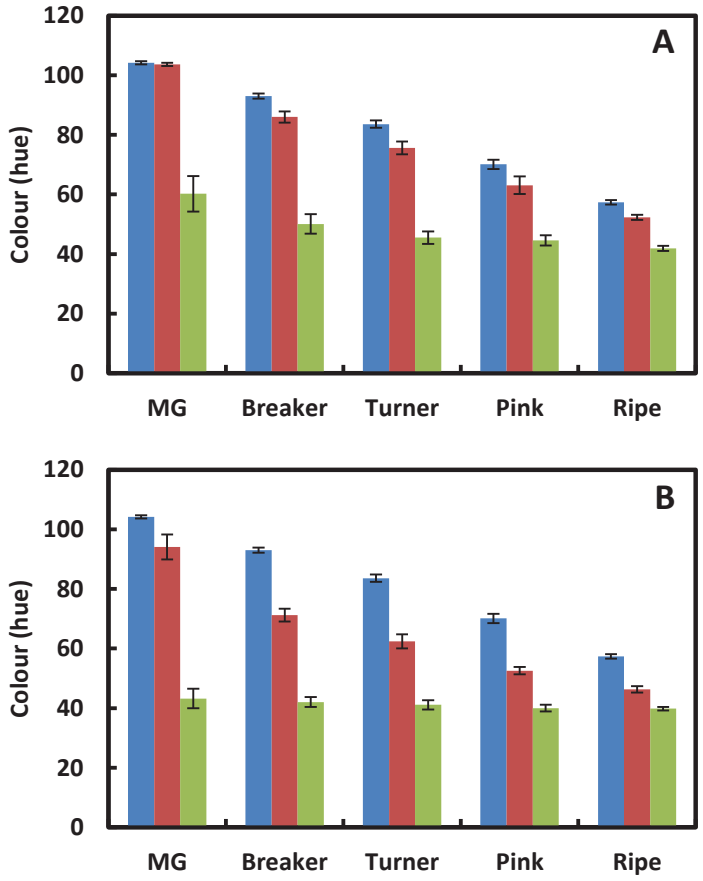

Figure 5.2 Hue angle of different maturity tomatoes at harvest (■) and after treated with (■) and without (■) 1-MCP and stored at 20 °C for 7 (A) and 14 d (B). Error bars represent SE (n = 12).

These results agree with previous work which has shown that the efficacy of 1-MCP is affected by fruit maturity (Hurr et al., 2005; Guillén et al., 2007) and the effect of 1-MCP in delaying or inhibiting ripening is greater in tomatoes harvested at the MG stage than pink stage (Mir et al., 2004; Sabir and Agar, 2011). Since sensitivity of tissue to ethylene is reduced by advancing maturity (Brady and Speirs, 1991), the inhibition of red colour development possibly declined with increasing fruit maturity.

Since 1-MCP blocks ethylene receptors and inhibits ethylene action (Sisler and Serek, 1997; Watkins, 2002), results reported here confirmed that ethylene perception was

required for both the initiation and progression of ripening in tomato (Hoeberichts et al., 2002) and supported the concept of ethylene being a regulator of, rather than a 'trigger' for fruit ripening (Theologis, 1992).

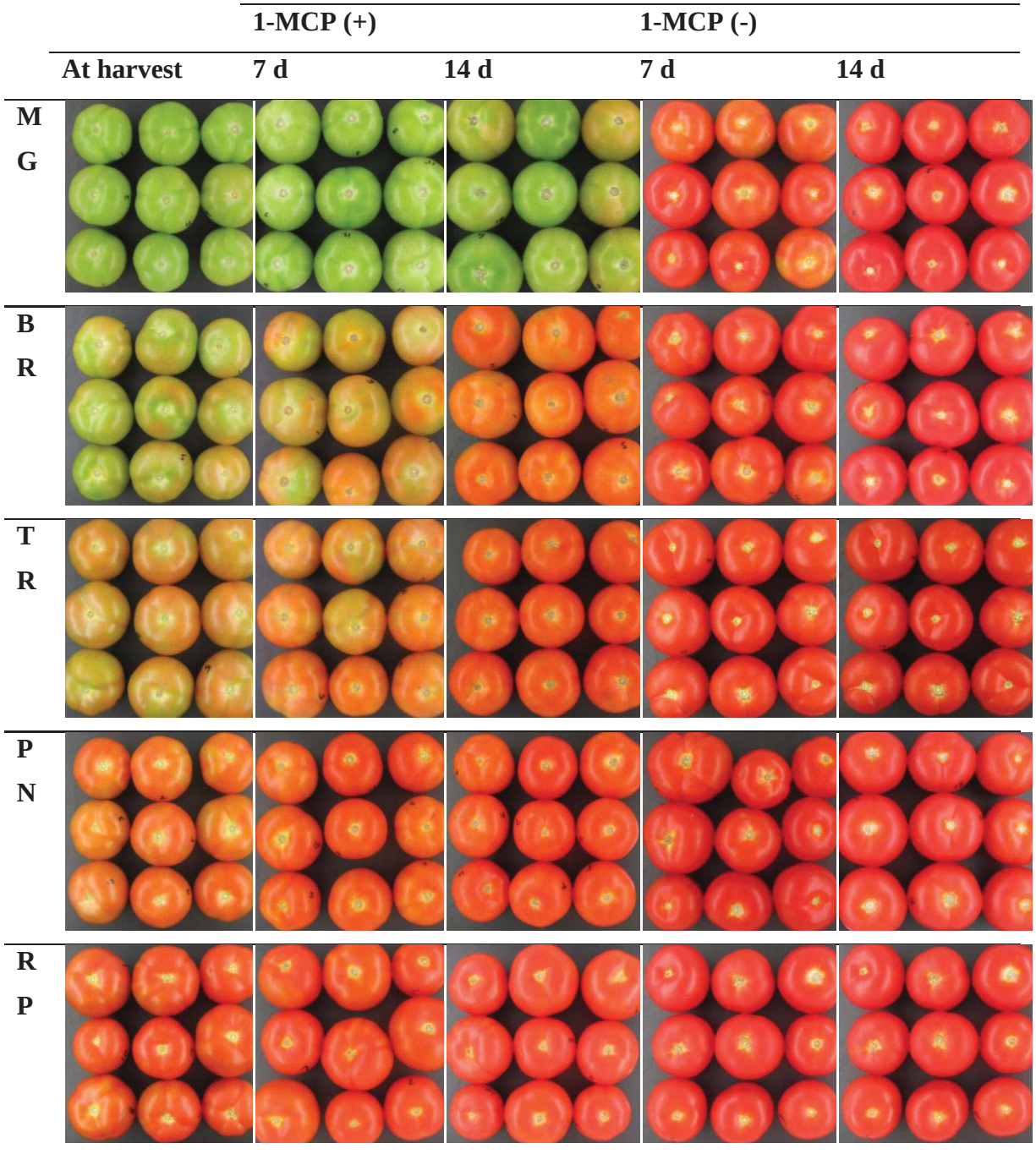

Figure 5.3 Visual appearance of different maturity (MG – mature-green, BR – breaker, TR – turner, PN – pink and RP – ripe) tomatoes treated with (+) and without (-) 1-MCP and ripened at 20 °C for 7 and 14 d.

5.3.2. Effect of 1-MCP on acoustic stiffness in tomatoes ripened at 20 °C

Fruit firmness changes during storage were influenced by 1-MCP treatment and the effects were strongly dependent on fruit maturity at the time of application. When 1-MCP treated fruit were stored for 7 d, loss of acoustic stiffness was highest in MG fruit as compared with fruit at advanced maturity stages (Figure 5.4A). While colour development of mature-green fruit was completely inhibited by 1-MCP for 7 d, fruit underwent significant softening under the influence of 1-MCP. Mir et al. (2004) reported that tomatoes treated with 1-MCP could decrease in fruit firmness without accompanying colour changes. Unlike red colour inhibition, efficacy of 1-MCP in delaying softening was increased with advancement of fruit maturity (Figure 5.4A).

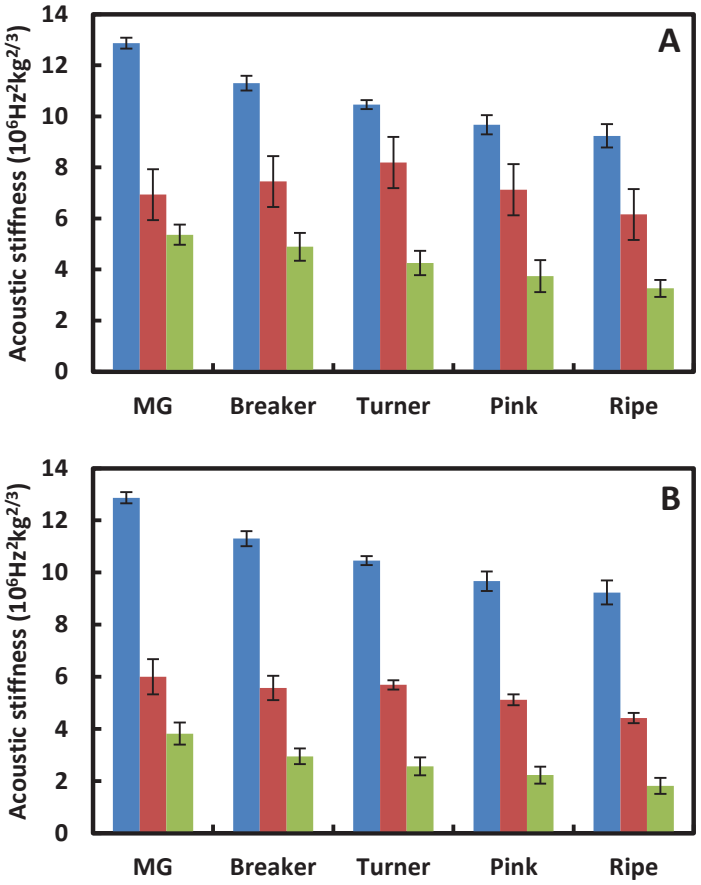

Figure 5.4 Acoustic stiffness of different maturity tomatoes at harvest (■) and after treated with (■) and without (■) 1-MCP and stored at 20 °C for 7 (A) and 14 d (B). Error bars represent SE (n = 12).

Compared to control, 1-MCP treated fruit of all maturities remained stiffer even after 14 d ($p < 0.05$, Figure 5.4B), indicating again ethylene perception was required even at advanced maturity stages for the coordinated completion of ripening. The ability of 1-MCP to retard or delay fruit softening has previously been reported in tomato (Mostofi et al., 2003; Guillén et al., 2006). The processes by which 1-MCP delayed softening could be related to regulation of enzyme activities (Wills and Ku, 2002). Mostofi et al. (2003) suggested that 1-MCP directly inhibited ethylene mediated changes in cell wall degrading enzyme activities, particularly PG (Carrington et al., 1993), β-galactosidases/galactanases (Sozzi et al., 1998) and PME (Brummell and Harpster, 2001). However, 1-MCP only delayed the rate of stiffness loss but not the initiation of fruit softening as tomatoes of all maturities lost stiffness significantly as compared to stiffness at-harvest after 7 d ($p < 0.05$). This could be explained by the fact that some components of softening are ethylene-independent (Leliévre et al., 1997). Other researchers have found evidence of ethylene-independent softening in climacteric fruit. For example in avocados, Jeong et al. (2003) reported that PG activity was strongly suppressed by 1-MCP, although softening did not require increased PG activity. In ACO antisense melons, accelerated fruit softening by exogenous ethylene was delayed upon removal of ethylene, though the softening was not entirely inhibited, suggesting that fruit softening depends only partially on ethylene (Flores et al., 2001). Kiwifruit softened significantly during low temperature storage independent of ethylene and 1-MCP failed to inhibit the softening (Mworia et al., 2012).

5.3.3. Effect of 1-MCP on colour development in tomatoes chilled at 2.5 °C

Skin colour development of both mature-green and breaker tomatoes was inhibited when fruit were treated with 1-MCP and stored at 2.5 °C for 35 d (Table 5.1). Mature-green fruit treated with 1-MCP remained green (hue = 101°) while non-treated control fruit showed a decrease in hue angle (91°) at the end of chilling period ($p < 0.05$). Breaker tomatoes followed the similar trend as mature-green fruit. By the end of 4 d post-chilling period at 20 °C, breaker tomatoes without 1-MCP were redder than fruit treated with 1-MCP (Table 5.1).

When 1-MCP treated mature-green tomatoes were stored at 20 °C for 35 d, fruit developed full red colour (hue = 40°) and no difference was found between 1-MCP treated and control fruit ($p > 0.05$, Table 5.1). Previously, it was observed that after 14 d of 1-MCP

treatment, mature-green tomatoes stored at 20 °C began to 'break' colour (Figure 5.2). Therefore, this result indicates that 1-MCP treated fruit eventually overcome the inhibition of ripening and develop full red colour at 20 °C when given sufficient time. Sisler et al. (1996) showed that 1-MCP binds irreversibly to ethylene receptors and suggested that fruit eventually overcome inhibition by making new receptors.

Table 5.1 Skin colour of mature-green (MG) and breaker (Brk) tomatoes treated with (+) or without (-) 1-MCP and stored at 2.5 or 20 °C for 35 d. Fruit were evaluated at the end of chilling at 2.5 °C and at the end of 4 d post-chilling period at 20 °C.

Treatments	End of Chilling at 2.5 °C	End of 4 d post-chilling at 20 °C
MG2.5 °C 1-MCP (+)	101.10 a	99.32 a
MG2.5 °C 1-MCP (-)	91.34 b	87.12 b
LSD α = 0.05	2.47	2.38
Brk2.5 °C 1-MCP (+)	82.80 a	81.57 a
Brk2.5 °C 1-MCP (-)	66.27 b	64.85 b
LSD α = 0.05	6.95	6.92
MG20 °C 1-MCP (+)	40.88 a	40.76 a
MG20 °C 1-MCP (-)	39.60 a	40.15 a
LSD α = 0.05	1.62	1.97

Pairs of values in columns followed by a different letter are significantly different at α = 0.05.

When 2.5 °C-stored fruit were transferred to 20 °C following 35 d of chilling, mature-green fruit treated with 1-MCP remained green and breaker fruit did not develop further red colour (Table 5.1). On the other hand, mature-green fruit without 1-MCP treatment developed yellow-orange colour (hue = 87°) possibly indicating advancement of ripening while breaker fruit without 1-MCP were redder than 1-MCP treated ($p < 0.05$ Table 5.1). In chapter 2, it was observed that mature-green 'Cedrico' tomatoes stored at 2.5 °C for 4 weeks lost the capacity to develop full red colour (Figure 2.1). Therefore, when MG and breaker fruit were treated with 1-MCP and stored at 2.5 °C for 35 d, colour development was inhibited even during a subsequent 4 d post-chilling period at 20 °C.

5.3.4. Effect of 1-MCP on acoustic stiffness in tomatoes chilled at 2.5 °C

Treatment with 1-MCP has previously been found to be effective in delaying firmness loss in tomato (Mostofi et al., 2003; Guillén et al., 2006). Unlike the effect of 1-MCP in delaying softening during ripening at 20 °C, 1-MCP treated mature-green tomatoes stored at 2.5 °C showed a similar loss of stiffness compared with the non-treated control fruit (Table 5.2). A significantly greater loss in stiffness was found when 1-MCP treated breaker tomatoes were stored at 2.5 °C compared to non-treated control ($p < 0.05$). This result indicates that 1-MCP affects softening during chilling differentially to normal ripening. Perhaps 1-MCP treatment rendered fruit more sensitive to chilling-induced softening by delaying ripening than non-treated control. Chilling-induced excessive softening has been reported in tomato (section 2.3.3; Efiuvwevwere and Thorne, 1988; Marangoni et al., 1995). It is possible that chilling has accelerated softening of both MG and breaker fruit and 1-MCP has exacerbated this (particularly in breaker fruit). However, fruit should be assessed periodically during storage to confirm the 1-MCP effect in future studies.

Table 5.2 Acoustic stiffness of mature-green (MG) and breaker (Brk) tomatoes treated with (+) or without (-) 1-MCP and stored at 2.5 or 20 °C for 35 d. Fruit were evaluated at the end of chilling at 2.5 °C and at the end of 4 d post-chilling period at 20 °C.

Treatments	End of Chilling at 2.5 °C	End of 4 d post-chilling at 20 °C
MG2.5 °C 1-MCP (+)	2.76 a	2.28 a
MG2.5 °C 1-MCP (-)	3.23 a	2.63 a
LSD α = 0.05	0.48	0.41
Brk2.5 °C 1-MCP (+)	1.37 b	0.99 a
Brk2.5 °C 1-MCP (-)	1.98 a	1.69 b
LSD α = 0.05	0.51	0.54
MG20 °C 1-MCP (+)	1.86 a	1.77 a
MG20 °C 1-MCP (-)	0.90 b	0.86 b
LSD α = 0.05	0.45	0.44

Pairs of values in columns followed by a different letter are significantly different at α = 0.05.

5.3.5. Effect of 1-MCP on decay severity in tomatoes chilled at 2.5 °C

Both treated and untreated tomatoes stored at 2.5 °C showed severe decay as fruit were stored for 35 d. For MG fruit, while no significant difference was apparent between treated and untreated fruit (Table 5.3); the problem seemed to be more aggravated in 1-MCP treated fruit (Figure 5.6). This was obvious for breaker tomatoes, where 1-MCP treated tomatoes showed higher decay severity than non-treated control fruit ($p < 0.05$; Table 5.3). On the other hand, when tomatoes were stored at 20 °C, the opposite trend was observed with 1-MCP reducing decay significantly ($p < 0.05$).

Table 5.3 Decay severity (0 - 4 scale) of mature-green (MG) and breaker (Brk) tomatoes treated with (+) or without (-) 1-MCP and stored at 2.5 or 20 °C for 35 d. Fruit were evaluated at the end of chilling at 2.5 °C and at the end of 4 d post-chilling period at 20 °C.

Treatments	End of Chilling at 2.5 °C	End of 4 d post-chilling at 20 °C
MG2.5 °C 1-MCP (+)	1.33 a	3.27 a
MG2.5 °C 1-MCP (-)	1.13 a	2.60 a
LSD α = 0.05	0.86	0.72
Brk2.5 °C 1-MCP (+)	2.30 a	3.40 a
Brk2.5 °C 1-MCP (-)	1.30 b	2.10 b
LSD α = 0.05	0.89	0.86
MG20 °C 1-MCP (+)	1.00 b	1.33 b
MG20 °C 1-MCP (-)	2.88 a	3.62 a
LSD α = 0.05	0.95	0.91

Pairs in values in columns followed by a different letter are significantly different at α = 0.05.

Reduction of decay by 1-MCP was possibly a function of storage temperature. At 2.5 °C, MG or breaker fruit were unable to advance in maturity rendering fruit more sensitive to chilling and thus developing more decay than non-treated fruit. On the other hand, 1-MCP treated fruit stored at 20 °C were possibly less ripe physiologically than fruit without 1-MCP and thus showed less decay since during normal ripening susceptibility to decay increases as tomato ripens (Fallik et al., 1993; Prusky, 1996). Therefore, the difference in 1-MCP effect on decay is due to the fact that 1-MCP shows differential response to chilling-induced decay and normal ripening-associated decay.

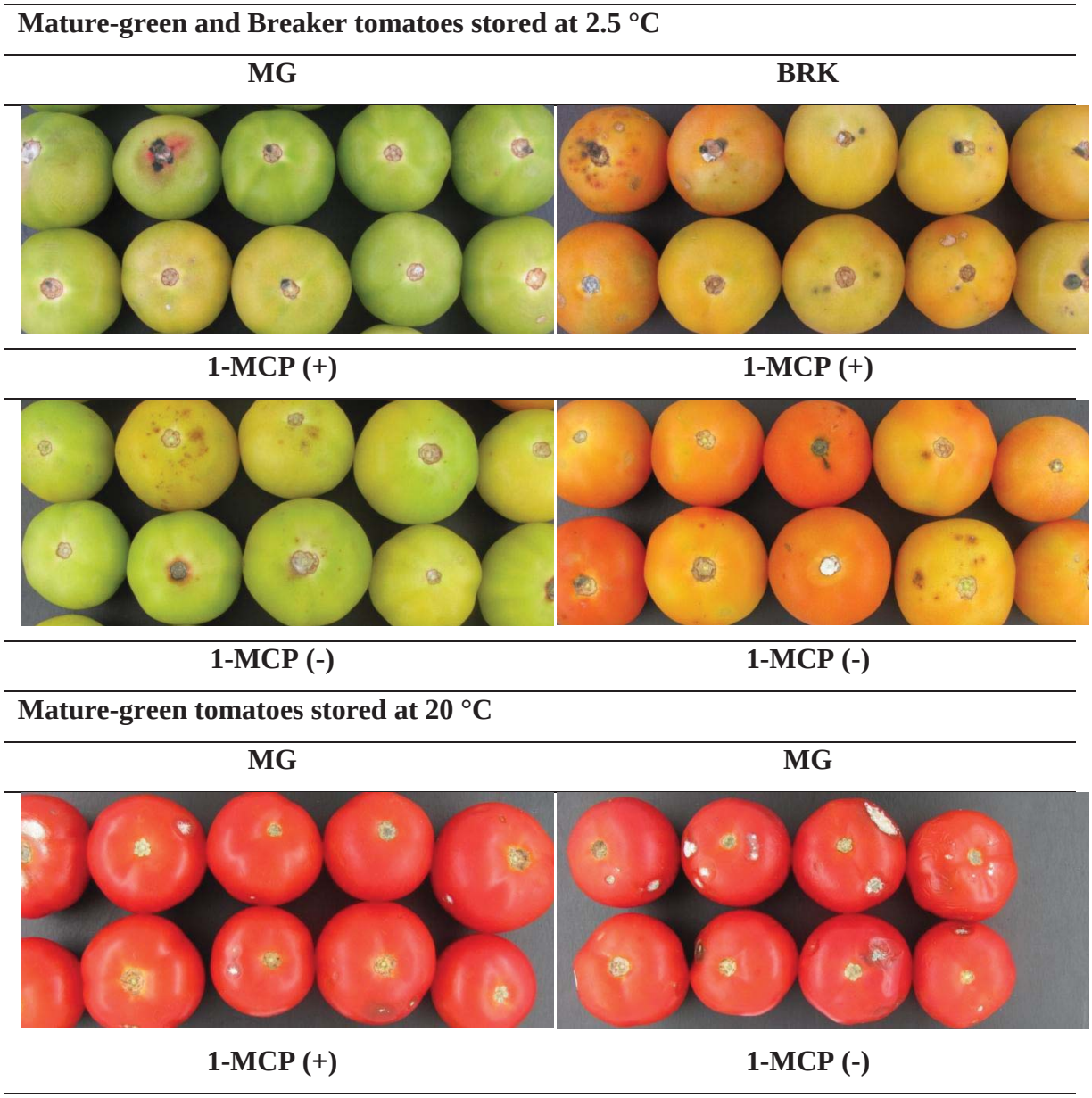

Figure 5.5 Visual appearance of mature-green (MG) and breaker (BRK) tomatoes treated with (+) and without (-) 1-MCP and stored at 2.5 or 20 °C for 35 d and examined immediately on removal to non-chilling temperature (20 °C).

It was possible that fruit stored at different temperatures became susceptible to different microorganisms. Area of decay development was quite different between storage temperatures. More than 80% of fruit stored at 2.5 °C developed decay at the calyx end which was characterised by black sunken spots attributed to *Alternaria* while none of the fruit stored at 20 °C developed decay on the calyx end either treated with or without 1-MCP (Figure 5.5 and Figure 5.6). Fruit stored at 20 °C showed white patches of decay

symptoms mostly on the surface of the fruit. These results confirm our previous observation in chapter 2 that entry of pathogens or area of decay development possibly depends on storage temperature and this could be due to the fact that different types of microorganisms attack fruit stored at different temperatures. Artés et al. (1993) speculated a close relationship between storage temperature and fungal rotting in lemon. They observed that lemons stored at 2 °C were mainly infected by *Alternaria citri* whereas those lemons stored at warmer temperature (20 °C) were rotten by *Penicillium digitatum* and *P. italicum*. In the present study, when fungi were isolated on potato dextrose agar and incubated for 72 h at 25 °C, light microscope has confirmed the presence of *Alternaria*, *Fusarium* in mature-green fruit and *Alternaria* and *Penicillium* in the breaker fruit stored at 2.5 °C. Some of the rot was developed by secondary fungi attack. Fruit stored at 20 °C were mostly infected with *Phytophthora* and *Rhizopus*.

Overall, our results suggest that 1-MCP can reduce or induce decay depending on storage temperature. The association of 1-MCP and fruit decay has been reported previously with mixed results. Guillén et al. (2006) found that 1-MCP was highly effective in reducing decay in many tomato cultivars harvested at advanced maturity stages (breaker or ripe) and stored at 10 °C, while Su and Gubler (2012) supported this where they found reduced decay in mature-green tomatoes stored at 18 °C. At 18 °C, fruit were allowed to ripen and 1-MCP reduced ripening associated decay. However, Jing and Zi-Sheng (2011) indicated that 1-MCP resulted in higher decay (chilling-induced) in mature-green tomatoes stored at 3 °C for 14 d than untreated control. It seems that the effect of 1-MCP in reducing decay depends on whether decay is associated with ripening or chilling-induced. It is possible that 1-MCP treatment accelerates decay in chilled fruit but not in non-chilled fruit and perhaps this may explain previous contradictory results.

Nonetheless, if ripening delay by 1-MCP resulted in increased chilling sensitivity, breaker tomatoes should have lower decay severity than MG fruit. But, no visual differences in decay severity were found between these two maturities fruit during post-chilling period at 20 °C. Perhaps fruit were stored at 2.5 °C for too long, so both maturities were equally prone to chilling induced decay. It was possible that fruit stored at 2.5 °C reached irreversible phase of CI by 35 d. Previously, it was demonstrated that ‘Cedrico’ tomatoes stored at 2.5 °C showed an increase in ion leakage after 2 weeks and fruit lost the capacity to develop red colour and increased decay severity after 4 weeks. Therefore, determining

the effect of 1-MCP in inducing chilling related decay development warrants further research with shorter storage periods and other chilling temperature ranges.

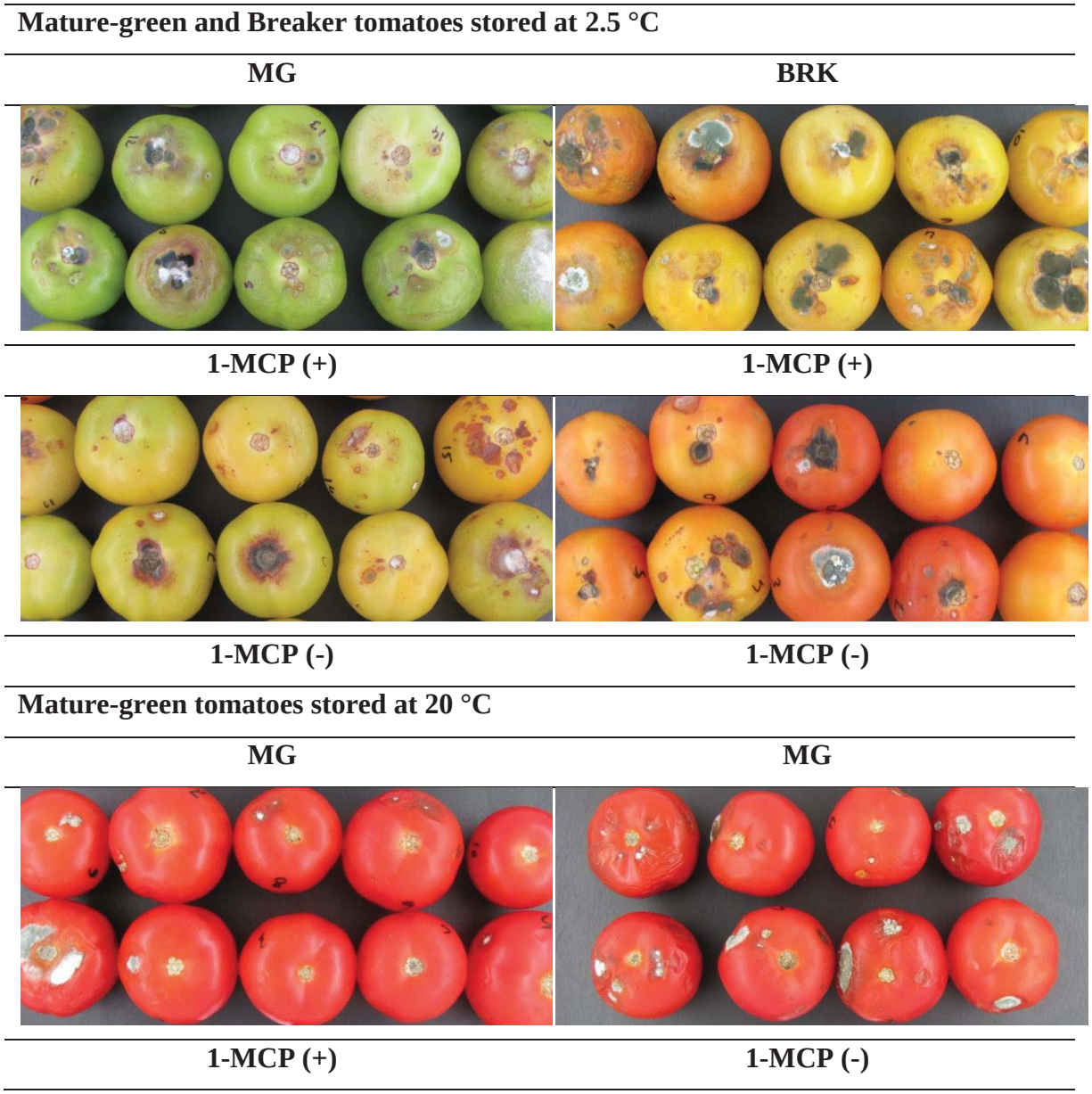

Figure 5.6 Visual appearance of mature-green (MG) and breaker (BRK) tomatoes treated with (+) and without (-) 1-MCP and stored at 2.5 or 20 °C for 35 d and examined 4 d after removal to non-chilling temperature (20 °C).

5.4. Conclusion

Effect of 1-MCP in delaying ripening of fruit is well documented. This chapter demonstrated that 1-MCP delayed colour development and retained firmness in tomatoes

during normal ripening, although this affect was influenced by fruit maturity at time of 1-MCP application. Application of 1-MCP can extend the storage life by inhibiting the advancement of ripening, although fruit eventually overcome the inhibition and resume ripening. Moreover, these results support the hypothesis that ethylene perception is required throughout ripening as shown by the effects on hue angle and fruit firmness.

The effect of 1-MCP on tomato ripening differs during chilling from normal ripening. During normal ripening 1-MCP retained stiffness and reduced decay whereas during low temperature storage more rapid loss of stiffness and increased decay was observed. Since 1-MCP inhibits advancement of fruit ripening, fruit may become more susceptible to CI and hence show more dramatic symptoms of CI than non-treated fruit. However, fruit had already spent too long at 2.5 °C to avoid chilling-induced *Alternaria* and that could explain why no significant differences in decay severity were seen in mature-green fruit. Therefore, it is important to investigate the effect of 1-MCP influencing decay susceptibility employing a range of low temperatures and varied duration of storage. Moreover, use of different maturity tomatoes needs to be considered in future research.

The effect of 1-MCP on postharvest decay in tomato is not consistent. Both increase or decrease decay incidence by 1-MCP has been reported in tomato. Although data are limited, results in this chapter provide an indication that 1-MCP enhances decay incidence in fruit stored at a temperature which causes chilling injury whereas it reduces decay when fruit are allowed to ripen normally. It is possible that in the former case, 1-MCP may have interfered with ethylene-induced resistance in ripening tomato fruit, while the later 1-MCP kept the fruit in the more resistant unripe condition. This provides one explanation of having mixed results of ethylene in influencing decay in tomato. Since 1-MCP can enhance chilling-induced decay, it is possible that ethylene may have positive results in reducing chilling-induced decay and thus will be discussed in next chapter. While 1-MCP shows promising results in extending postharvest storage life in many crops including tomatoes, our results and others (Jing and Zi-Sheng, 2011) indicated that application of 1-MCP may not be appropriate for commercial use in tomatoes before cool storage.

6. Intermittent warming-stimulated ethylene reduces tomato chilling injury

6.1. Introduction

An inter-relationship between ethylene production and CI has been proposed (Wang, 1989). However, the role of ethylene in development of CI is complex (Watkins and Ekman, 2004) as ethylene treatment can reduce, increase or have no effect at all on CI development (Wang, 1989). More importantly, ethylene may influence chilling sensitivity of tissues but not necessarily increase or reduce all types of symptoms in a given tissue. Since chilling injury is a collective term for a set of physiological disorders found in chilling sensitive tissues, the effects of ethylene may be different for different symptoms/tissues. Kader and Morris (1975) reported that exposure of mature-green tomatoes to ethylene or ethephon before storage at 0 or 5 °C reduced abnormal ripening but not the severity of other chilling symptoms during post-storage ripening at 20 °C. In peaches, application of ethylene during cool storage reduced woolliness but significantly enhanced fruit decay and pulp softening (Girardi et al., 2005). Exposure of sweet potatoes to ethylene for 2 d at 20 °C following storage at 1 °C for 2-6 d reduced CI severity as indicated by "hardcore" in the roots but ethylene had an adverse effect on flavour and colour (Buescher, 1977). Therefore for each fruit or tissue type, it is important to specify whether induction or reduction of particular chilling symptoms could be ethylene dependent or independent.

Failure to ripen, uneven blotchy red colouration, and increased susceptibility to decay have been reported as CI symptoms in tomatoes (Chapter 2). Excessive softening following chilling (which can be confounded by ripening-associated softening) was also perceived as a CI symptom in tomatoes (2.3.3). It is generally accepted that chilling sensitivity in tomato reduces as fruit ripen (2.3.4.2; Autio and Bramlage, 1986) and that this could be related in some way to the advancement of climacteric ethylene production. However, it is not clear whether reduction of chilling sensitivity is independent of simple ethylene-induced ripening. Saltveit and Morris (1990) argued that ripe fruit may appear more chilling-resistant simply because they cannot exhibit alterations in the already accomplished process of ripening (i.e. red colour development). In mango, mature-green fruit with adequate ethylene production could initiate ripening and avoid CI, but immature

fruit had lower ethylene concentrations insufficient to initiate ripening and developed CI symptoms (Mohammed and Brecht, 2002). Barden and Bramlage (1994) found that greater maturity and ripening of fruit on the tree was generally associated with less superficial scald during storage of apple at 0 °C. Similarly, pre-climacteric fruit were more sensitive to CI than post-climacteric fruit in avocados (Kosiyachinda and Young, 1976), honeydew melons (Lipton, 1978), and papayas (Chen and Paull, 1986). Moreover, reduction of woolliness in nectarines by delayed storage for 2 d at 20 °C before placing fruit at 0 °C was attributed to commencement of initial ripening processes that continued during cool storage (Zhou et al., 2000).

Intermittent warming (IW) alleviated CI in tomato (Chapter 4; Artés and Escriche, 1994). The mechanism(s) by which IW reduces CI is not clear (Sevillano et al., 2009). In tomato, IW has been observed to stimulate ethylene production during and subsequent to each warming occasion (Artés et al., 1998a; Biswas et al., 2010) and this response coincided with advancing respiratory climacteric peak and red colour development (Biswas et al., 2010). In chapter 4, it was demonstrated that three cycles of IW to 20 °C for 1 d after every 6 d at 6 °C led to higher ethylene production than in fruit without IW (Figure 4.3). In contrast, fruit chilled continuously did not produce detectable ethylene and developed uneven, blotchy colouration. Advanced red colour development of IW fruit during low temperature storage could be attributed to stimulated ethylene production. In other postharvest warming treatments such as hot water treatment, increased ethylene evolution associated with enhanced red colour development has been reported for tomato (Soto-Zamora et al., 2005). However, it remained unclear whether advanced ripening by heat treatment was a cause or consequence of stimulated ethylene production.

In peaches, IW maintains fruit quality and reduces woolliness by inducing production of enough ethylene to allow ripening (Fernández-Trujillo et al., 1998). In peaches stored at 0 °C, mRNA of ACS and ACO decreased while IW to 20 °C for 24 h induced transcription of these two genes and stimulated conversion of ACC to ethylene (Zhou et al., 2001). The authors demonstrated that IW stimulated ethylene production, assisting fruit to ripen normally and reduce woolliness. In support of this observation, Dong et al. (2001) demonstrated that inhibition of ethylene action with 1-MCP enhanced incidence of woolliness, consistent with increased ethylene reducing CI in peaches. Alwan and Watkins

(1999) reported that IW resulted in greater ethylene production than control and reduced superficial scald in apples stored at 0.5 °C by advancing fruit ripening.

Although limited, these works suggest that increased ethylene plays a role in the reduced chilling injury effects observed for IW treatments during low temperature storage. Ethylene may also help tomatoes to ripen properly and reduce CI. It has already been established that IW of tomatoes stored at low temperatures stimulates ethylene production and results in red colour development and increased chilling tolerance. What is not established is whether this stimulated ethylene during IW is just a correlation with red colour development or a cause of advanced maturation in tomatoes. The objective of this chapter was to investigate the role of IW-stimulated ethylene in reducing CI in 'Cedrico' tomatoes stored at 6 or 2.5 °C. Elimination of ethylene responses was achieved through application of 1-MCP, an ethylene action inhibitor. Alternatively, exogenous ethylene (100 $\mu L.L^{-1}$) was applied to tomatoes during low temperature storage to determine the role of ethylene independently of increased temperature (IW). Ethylene has been applied to tomatoes before or after cool storage (Chomchalow et al., 2002). However, to our knowledge, this is the first investigation of ethylene being applied intermittently for 24 h after every 6 d during cool storage to investigate whether intermittent ethylene (IE) supply can be used as an alternative approach to IW for reducing CI in tomato. The result of this work would provide insight not only into the role of ethylene in reducing chilling injury in tomato, but potentially inform about possible mechanisms of IW as a technology, and may contribute to identifying new methodologies adaptable to industry that may assist in reduction of chilling injury risk during prolonged low temperature storage.

6.2. Materials and methods

Two storage experiments (6 or 2.5 °C) were experimentally carried out sequentially due to equipment and availability of storage room constraints. Additionally, there was a limitation of obtaining large number of single maturity tomatoes (mature-green) at a time.

6.2.1. Fruit

Tomatoes ('Cedrico') were grown in a Massey University greenhouse and harvested at mature-green stage. After harvest, fruit were transferred immediately to 20 °C and left over night. Fruit growing conditions were as described in chapter 2 (section 2.2.1).

6.2.2. Storage conditions

Fruit calyces were carefully removed and a total of 300 fruit were randomly selected for each storage experiment (5 treatments x 3 replications x 5 fruit in each replication x 4 destructive measurement times). The fruit were placed inside polyvinyl chloride (PVC) tubes with flow-through system supplying humidified air (around 95% RH) at a total flow of 200 mL.min^{-1} and held at 6 or 2.5 °C for 27 d. At each storage temperature (6 or 2.5 °C) there were 5 treatments.

The treatments were:

1. Constant cool storage at 6 or 2.5 °C (Control)

2. Intermittent ethylene (100 μL.L^{-1} C_2H_4, 21% O_2 in nitrogen) application every 7 d for 24 h for fruit stored at either 6 or 2.5 °C (6 °C+IE or 2.5 °C+IE)

3. Intermittently warming for fruit stored at either 6 or 2.5 °C every 7 d to 20 °C for 24 h (IW: 6 d 6 °C + 1 d 20 °C or 6 d 2.5 °C + 1 d 20 °C)

4. Intermittently warming with intermittent ethylene supply for 24 h during each warming period (IW+IE) (both for 6 and 2.5 °C storage temperature)

5. Intermittent warming after pre-storage treatment with 5 μL.L^{-1} 1-MCP (IW+1-MCP) (both for 6 and 2.5 °C storage temperature)

Fruit physiology (ethylene production and respiration rate) and quality (chilling injury severity, colour, and firmness) were evaluated during 27 d of storage and a subsequent 7-10 d post-chilling period. Some fruit were also transferred to 20 °C after 13 d of storage with only CI severity determined after a subsequent 7-10 d. IW treatment was achieved as described previously (section 4.2.1).

6.2.2.1. Ethylene gas exposure

Ethylene treatment was conducted during refrigerated storage at 6 or 2.5 °C and during intermittent warming conditions for 24 h via a flow-through system supplying 100 μL.L^{-1} ethylene in humidified air at a flow of 200 mL.min^{-1} (Figure 6.1). The desired

concentration of ethylene was obtained by using an ethylene gas cylinder containing β-standard 100 $\mu L.L^{-1}$ C_2H_4, 21% O_2 in nitrogen (BOC, New Zealand). Ethylene and air were connected to the manifold and then the required gas connected to each PVC tube (volume = 0.0135 m^3). All gas was bubbled through flasks (placed in the storage room) containing 500 mL of 21% glycerol in water to humidify the gas to around 95% RH before continuing to each PVC tube. The proportion of water and glycerol needed to generate a required RH was calculated using the equation provided by Forney and Brandl (1992).

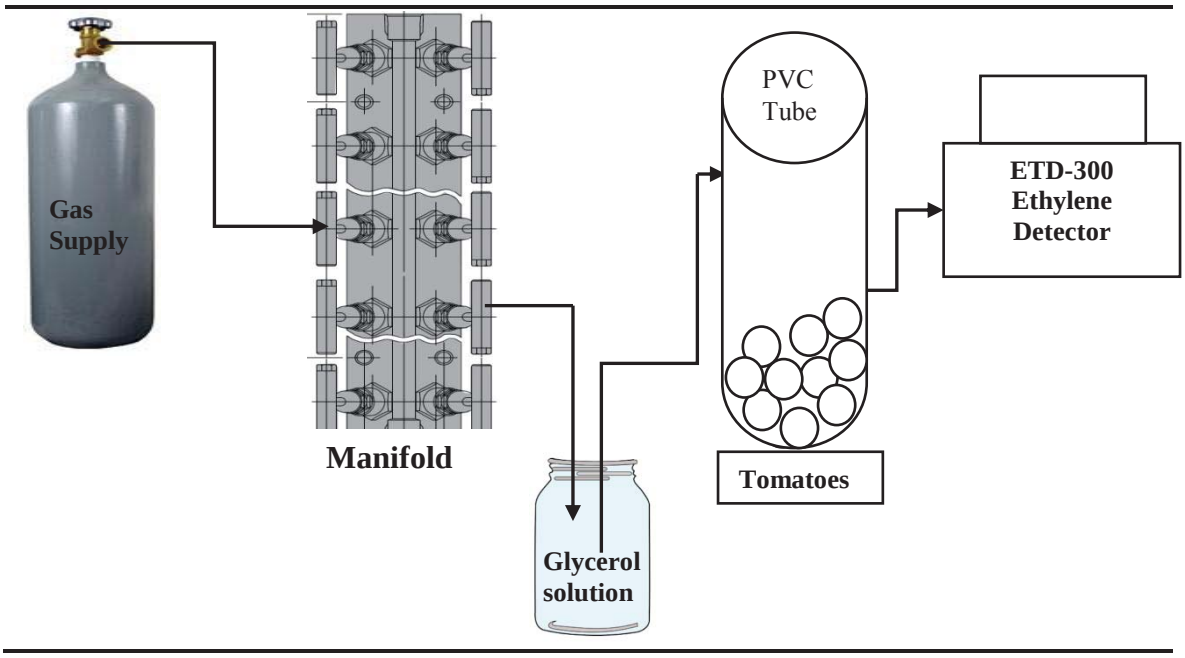

Figure 6.1 Graphical view of gas supply (ethylene or air) to the fruit kept in PVC tube.

6.2.2.2. 1-MCP treatment

For each experiment (6 or 2.5 °C), 60 fruit were placed in two 56 L airtight sealed containers (30 fruit each container) for 24 h at 20 °C and treated with 5 $\mu L.L^{-1}$ of 1-MCP (refer section 5.2.2). The non-treated fruit were held in an identical sealed container (8 containers with 30 fruit each) with air for 24 h at 20 °C. After 24 h treatment, the sealed containers were opened outdoors and fruit were placed in the PVC tubes.

6.2.3. Quality Assessment

6.2.3.1. CI severity

In this chapter, CI symptoms were expressed as shrivel, rot, and blotchy red colouration. CI symptoms were assessed using 0 to 4 visual rating scales and average severity was calculated as described in section 2.2.5.

6.2.3.2. Surface colour

Colour was measured by a reflectance spectrophotometer (CM-2600D, Konica Minolta Sensing Inc., Japan). Details are described in section 2.2.2.

6.2.3.3. Fruit firmness

Previously firmness of tomatoes was measured by acoustic stiffness and compression force (Chapter 2). Acoustic stiffness and compression force are largely a measure of mechanical stiffness of tissue which is based on both the cell wall mechanical strength and tension under which tissue is held by turgor (Hertog et al., 2004). Given that many factors influence the firmness of chilling-injured tomato and different measurement methods will indicate different characteristics of tomato firmness (Jackman et al., 1990), the present study employed invasive puncture force along with acoustic and compression force. However, the puncture from outside of whole fruit is largely influenced by skin toughness. Therefore, a puncture test from the inside of an excised pericarp disc was performed to ensure measurement of pericarp firmness alone.

Non-invasive acoustic response and compression force was determined as previously detailed in section 2.2.4. Puncture test was performed using a flat end cylindrical probe (3.7 mm diameter) mounted on a TA-XT plus texture analyser (Stable Micro Systems Ltd.). The test was run using a pre-test and a test speed both of 10 mm s^{-1}, a trigger force of 0.1 N, and allowing the probe to travel 15 mm deep into the tissue, measuring the maximum force encountered. For puncture from inside of a pericarp disc, equatorial discs were cut with a 15 mm diameter cork borer and locular tissue was carefully removed. A flat end cylindrical probe (3.7 mm diameter) was used to puncture the disc tissue to a depth of 4 mm using same test run protocol as puncture of whole fruit. From each fruit two discs were made and each disc was punctured at two places.

6.2.4. Fruit physiology assessment

6.2.4.1. Respiration rate

Respiration rate was measured as the production of carbon dioxide (CO_2). As fruit placed in the sealed PVC tube were supplied with continuous air flow, it can be assumed that the internal atmosphere of the tube was in a state of equilibrium. A 1 mL gas sample was collected from the tube. The concentration of CO_2 in the 1 mL gas sample was measured with a miniature infrared CO_2 transducer (Analytical Development Co, Hoddesdon, UK), using N_2 as carrier gas at 35 $mL.min^{-1}$. The equipment was calibrated with β-standard 0.49 ± 0.01% CO_2 (BOC, New Zealand). Output signals were linear over the range analysed and recorded with an HP 3396A (Hewlett Packard, USA) integrator. Individual tube outflow was measured and the rate was used for respiration rate ($mol.kg^{-1}.s^{-1}$) using following equation.

$$\text{Production Rate} = \frac{\left(C \times G_{Fl}\right) \times P \times 10^{-2}}{R.T.m}$$

Where, C = CO_2 concentration (%); G_{Fl} = gas flow rate ($m^3.s^{-1}$); m = fruit mass (kg); P = atmospheric pressure (Pa); R = universal gas constant (8.314 $Pa.m^3.mol^{-1}K^{-1}$); T = temperature (K), 10^{-2} = conversion factor (percentage to mol fraction).

6.2.4.2. Ethylene production

Ethylene production of fruit held in PVC tube was measured with an ETD-300 ethylene detector (Sensor Sense, Nijmegen, Netherlands). This ethylene sensor detector provides an opportunity to measure ethylene at very low concentrations (nominally 300 $nL.L^{-1}$). Air was flowed consistently to the tomatoes placed inside PVC tubes and the ethylene concentration of the outflow was measured (Figure 6.2). Since flow rates through "flow through" experiments tended to exceed the maximum flow through the ETD-300 (5 $L.h^{-1}$), the outlet flow could not be directly sampled. A solution was to fit a T-junction fitted with a sampling septum on the outlet flow and sampled from this location with an in-line suction pump (Micro Diaphragm pumps, 6 volt, NMP 05B, Hivac Ltd, New Zealand). Directing the flow generated from this pump into the ETD-300 inlet and using a closed channel loop allowed ethylene concentration analysis of this flow.

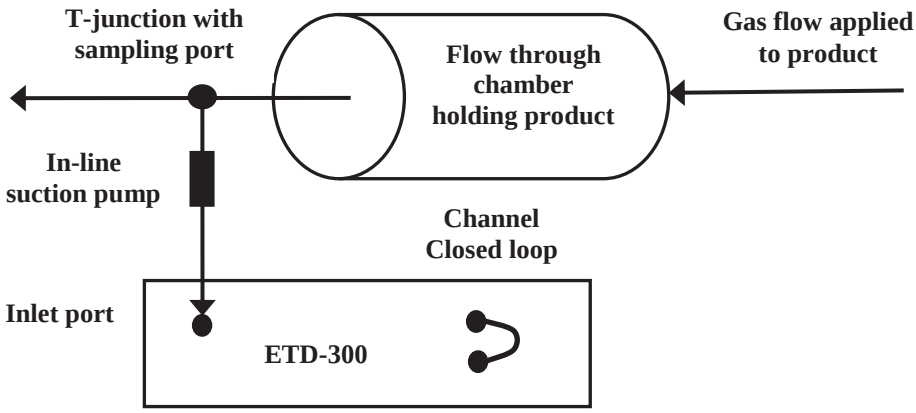

Figure 6.2 Schematic view of outflow sampling method for measuring ethylene concentration by ETD-300 using continuous flow-through method.

In continuous flow method, with the knowledge of the mass of product and the flow rate of the applied gas, measurement of the ethylene concentration in the gas outlet allows measurement of the ethylene production. Ethylene production rate ($mol.kg^{-1}.s^{-1}$) was calculated using following equation:

$$\text{Production Rate} = \frac{(E \times G_{Fl}) \times P \times 10^{-9}}{R.T.m}$$

Where, E = ethylene concentration (ppb); G_{Fl} = gas flow rate ($m^3.s^{-1}$); m = fruit mass (kg); P = atmospheric pressure (Pa); R = universal gas constant (8.314 $Pa.m^3.mol^{-1}K^{-1}$); T = temperature (K), 10^{-9} = conversion factor ($\mu L.L^{-1}$ to mol fraction).

During the storage experiment at 2.5 °C, ETD-300 ethylene sensor broke down after 17 d of storage. No ethylene production was measured after 17 d.

6.2.5. Statistical analysis

A complete randomized design was used for this study. Effect of treatments was analysed using the General Linear Model procedure of SAS (SAS Institute, version 9.2, Cary, NC). When appropriate, means of different treatments were compared using Fisher's least significant difference at the 5% level.

6.3. Results

Two separate experiments were conducted at two different storage temperatures and results will be discussed separately as part A (6 °C) and part B (2.5 °C).

6.3.1. Part A – 6 °C storage

6.3.1.1. CI severity

Different kinds of CI symptoms were observed when fruit were stored at 6 °C for 13 or 27 d and subsequently transferred to 20 °C for 10 d (Table 6.1 and Table 6.2). After 13 d storage, fruit stored at constant 6 °C showed blotchy red colouration (Table 6.1) and almost 30% fruit remained green (Figure 6.3A). Fruit stored at 6 °C exposed to one cycle of ethylene (6 °C+IE) did not show comparative advantage as fruit also showed blotchy colouration. For the 13 d storage, one cycle of IW was achieved. One cycle of IW or IW in combination with ethylene (IW+IE) advanced red colouration of fruit and reduced CI but did not eliminate all symptoms. When IW fruit were treated with 1-MCP to block ethylene perception, IW+1-MCP fruit showed blotchy red colouration after storage at 20 °C (Table 6.1).

Table 6.1 Mean of CI severities (0 - 4 scale) of mature-green tomatoes after 10 d post-storage period at 20 °C preceded by 13 d chilling temperature (n = 15).

Treatments	Shrivel	Rot	Blotchy colour
6 °C	0.27 b	0.13 a	0.87 bc
6 °C + IE	0.33 b	0.07 a	1.40 b
IW	0.20 b	0.00 a	0.67 bc
IW + IE	0.27 b	0.00 a	0.33 c
IW + 1-MCP	2.20 a	0.00 a	2.33 a

Mean separation within columns by LSD at $\alpha = 0.05$.

Chilling sensitivity increases with extension of low temperature storage duration (Chomchalow et al., 2002). When exposure to 6 °C was extended to 27 d and fruit were subsequently examined after 10 d at 20 °C, overall CI severity had increased 3-fold in tomatoes which were held previously at constant 6 °C and these fruit had highest CI incidence and severity amongst treatments (Table 6.1 and Table 6.2). Importantly, this was the first time rot incidence in some fruit were observed in ‘Cedrico’ tomatoes held at 6 °C.

This may have been a result of maintaining a relative humidity higher than 95% constantly inside the PVC tube with a continuous flow of humidified air whereas previously fruit were kept in a cardboard box containing a single layer polyethylene liner where 90-95% RH was maintained (2.2.1). Applying ethylene in 6 °C-stored fruit (6 °C+IE) reduced CI (Table 6.2). Three cycles of IW with or without exogenous ethylene significantly alleviated CI in comparison with storage at continuous 6 °C ($p < 0.05$). Blocking ethylene response of IW fruit by 1-MCP resulted in failure to develop full red colour and fruit became orange in colour (Figure 6.3B). For the 13 d storage, IW+1-MCP treated fruit were yellow-orange in colour. These results suggest a role of ethylene in developing red colour in IW fruit. However, preventing ethylene response by 1-MCP did not eliminate all beneficial effect of IW; since IW+1-MCP reduced rot suggesting some ethylene-independent benefit of IW.

Table 6.2 Mean of CI severities (0 - 4 scale) of mature-green tomatoes after 10 d post-storage period at 20 °C preceded by 27 d chilling temperature (n = 15).

Treatments	Shrivel	Rot	Blotchy colour
6 °C	0.27 b	0.93 a	3.13 a
6 °C + IE	0.00 b	0.13 b	0.47 b
IW	0.00 b	0.00 b	0.00 b
IW + IE	0.13 b	0.00 b	0.00 b
IW + 1-MCP	2.67 a	0.13 b	0.53 b

Mean separation within columns by LSD at $\alpha = 0.05$.

1-MCP treated fruit showed highest shrivel for both 13 and 27 d storage (Table 6.1 and Table 6.2). Uneven surface contours arising from cell collapse, desiccation and shrivel have been previously reported for 1-MCP treated tomatoes (Hurr et al., 2005). Uneven surface contour due to cell separation was also reported as a CI symptom in tomatoes stored at 5 °C for 2-4 weeks (Hobson, 1987; Ding et al., 2001). In the present study, however, shrivel was not apparent in chilled fruit instead being largely found in 1-MCP treated fruit indicating shrivel in IW+1-MCP treated fruit was possibly not chilling-induced. Perhaps higher concentration of 1-MCP (5 $\mu L.L^{-1}$) has some toxic effect on fruit surface which has not been previously reported but few researchers have used this high concentration.

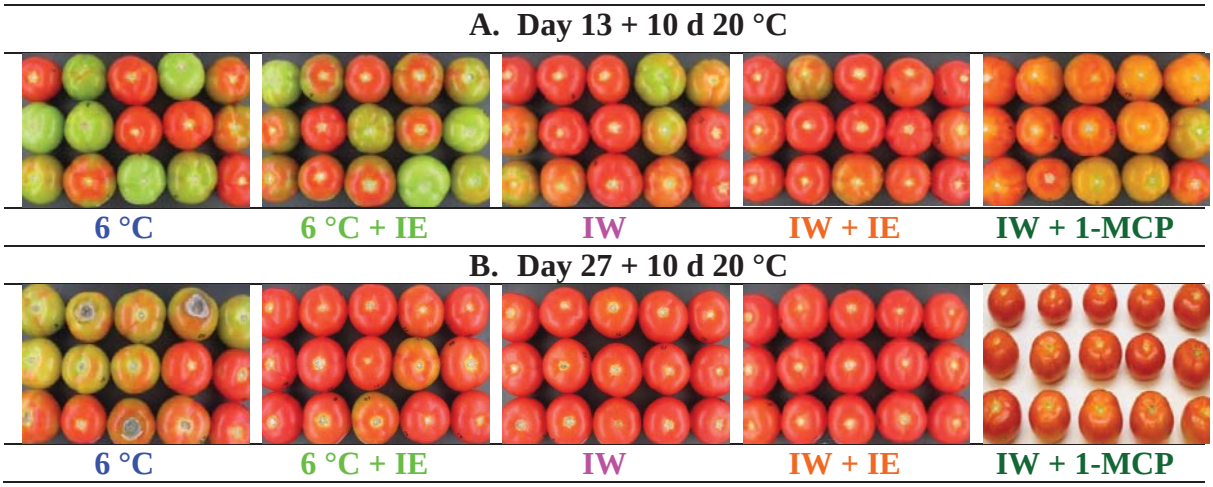

Figure 6.3 Visual appearance of mature-green tomatoes during post-storage period at 20 °C for 10 d following 13 (A) or 27 d (B) of chilling at 6 °C.

Overall, CI severity increased with extended duration at 6 °C. IW reduced CI significantly but effectiveness depended on number of warming cycles. One cycle of warming was inadequate to show a beneficial effect on red colour development in cool-stored tomatoes. Alternatively, exogenous ethylene application to fruit stored at 6 °C reduced CI significantly although one cycle of ethylene application was not enough. IW fruit treated with 1-MCP remained green before post-chilling removal to 20 °C and fruit became yellow-orange in colour at the end of post-chilling period, indicating blocking ethylene response disturbed red colour development in IW fruit.

6.3.1.2. Surface colour

Tomatoes stored at constant 6 °C for 27 d did not turn red in storage and developed uneven blotchy red colouration after storage at 20 °C (hue = 75°; Figure 6.4). Fruit at 6 °C+IE did not change colour in storage (hue = 101°). However, during post-storage period at 20 °C, more than 80% of those ethylene treated fruit that had been maintained at 6 °C subsequently developed full red colour. Researchers have suggested that during cool storage ethylene binding capacity of tissue decreases (Jiang et al., 2004) or tissue is insensitive to respond to ethylene (Ogura et al., 1976). However, result reported here suggests that tissue perceived ethylene during storage at 6 °C but phenotypic changes only appeared once fruit were moved to higher temperature (20 °C).

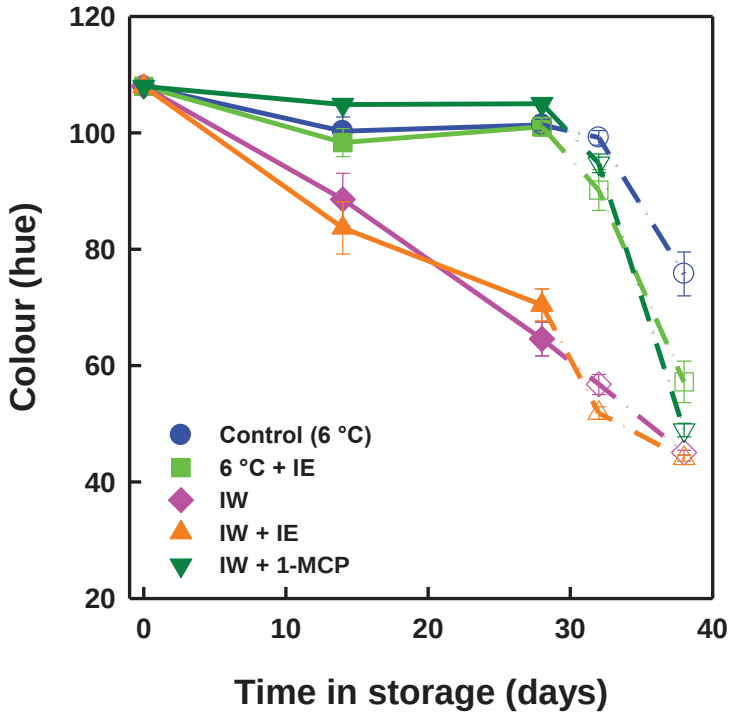

Figure 6.4 Surface colour of mature-green tomatoes held under different treatment conditions for 27 d at 6 °C. Dashed lines with open symbols indicate post-chilling transfer of fruit to 20 °C. Error bars represent the standard error (n = 15).

Tomatoes subjected to IW alone or IW with ethylene (IW+IE) developed red colour as indicated by decreasing hue in storage and hue value decreased further during post-storage period at 20 °C (hue ≈ 45°). In contrast, fruit treated with 1-MCP and submitted to IW (IW+1-MCP) had a hue value 105° and fruit were green in storage at 6 °C suggesting ethylene response played a role in advancing red colour development for IW fruit. Those 1-MCP treated fruit, however, eventually developed yellow-orange colour as indicated by reduced hue on their removal to 20 °C (Figure 6.4).

6.3.1.3. Fruit firmness

In the present study fruit firmness was determined by four different measurement techniques. The firmness of tomatoes decreased progressively in all treatments (Figure 6.5). Both chilling and ripening can contribute to fruit softening during postharvest storage but the degree and mechanism of softening due to these two physiological processes are quite different (Almeida and Huber, 2008). Therefore, it is important to differentiate softening induced by chilling from ripening-associated softening. Here, firmness data were

plotted against colour (an indicator of ripening) to investigate the relationship in the changes of these two quality indices as a function of storage treatment.

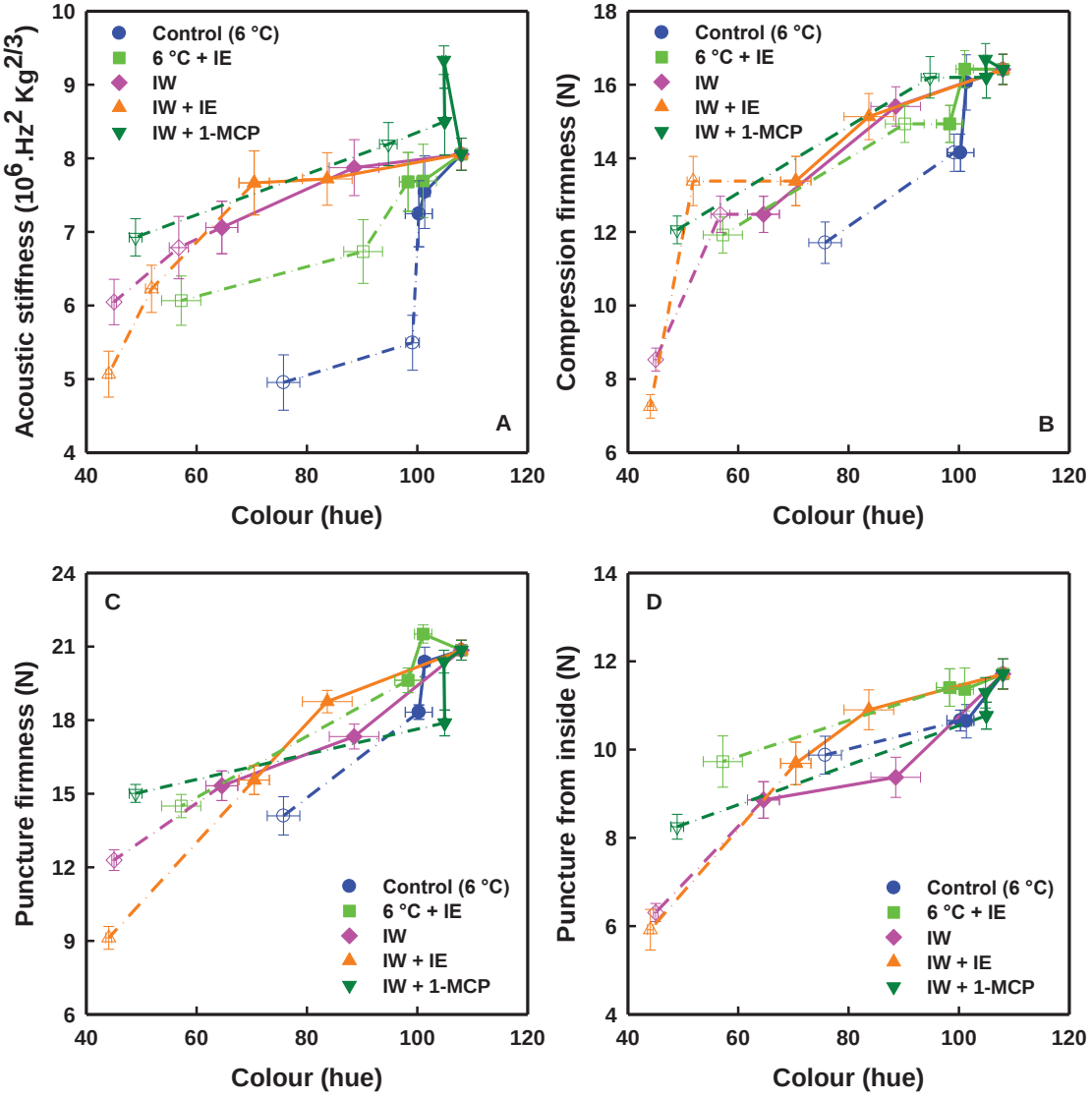

Figure 6.5 Relationship between colour changes (hue) and firmness of mature-green tomatoes held at different treatment conditions. Firmness was measured by acoustic sensor (A) compression force (B), puncture force (C) and puncture from inside of a pericarp disc (D). Dashed lines with open symbols indicate post-chilling transfer of fruit to 20 °C. Error bars represent the standard error (n = 15).

When firmness was measured by acoustic sensor measurement, which mainly measures tissue turgor, control fruit (6 °C) showed a different trajectory in stiffness loss as compared to fruit subjected to IW (Figure 6.5A). Storage at constant 6 °C decoupled stiffness loss

from colour changes (ripening) whereas fruit exposed to IW with or without exogenous ethylene coordinated softening with colour change simultaneously during post-chilling period at 20 °C. This observation mimicked our previous results where stiffness loss in fruit stored at constant 6 °C and fruit subjected to IW was attributed to chilling and ripening respectively (Chapter 4, Figure 4.7). Importantly, when fruit at constant 6 °C were treated with intermittent ethylene, 6 °C+IE treatment restored the ripening coordination in terms of softening and colour change during post-chilling removal to 20 °C. Overall, 6 °C+IE remained stiffer than control fruit ($p < 0.05$). If storage at constant 6 °C resulted in chilling-induced turgor loss, it seems that ethylene treatment allowed those fruit to retain higher stiffness at a comparable measurement time, indicating a protective mechanism of ethylene from chill-induced excessive softening. IW fruit treated with 1-MCP (IW+1-MCP) turned yellow-orange in colour after storage at 20 °C and followed the same path in stiffness loss as IW alone. However, IW influenced these two ripening processes before post-chilling removal to 20 °C, whereas IW+1-MCP treatment changed these processes mainly after transfer to 20 °C (Figure 6.5A).

Both compression firmness and puncture test measurement followed largely a similar trend as acoustic stiffness measurement in all treatments (Figure 6.5B and C). However, the change in trajectory of firmness loss for control fruit at constant 6 °C was less dramatic when measured with these techniques than acoustic sensor measurement. Meanwhile, at the end of post-chilling period at 20 °C, effect of ethylene on fruit firmness was obvious when ethylene was applied during IW period. IW fruit treated with ethylene (IW+IE) were softer than IW fruit without ethylene. In contrast, when ethylene response of IW fruit was blocked by 1-MCP, IW+1-MCP fruit were firmer than fruit with IW alone. Therefore, effect of 1-MCP and ethylene on IW fruit are consistent in hypothesis that IW-induced ethylene accelerated colour development and softening.

When fruit firmness was determined by invasive puncture force from outside of epidermis, fruit skin may contribute to an error in perception of actual chilling-induced softening. Therefore, puncture test was also performed from inside of a pericarp disc to determine tissue integrity. When puncture was performed from inside of an excised pericarp disc, at-harvest firmness values were two times less than that obtained from puncture from outside epidermis of whole fruit indicating fruit skin played a part to make this difference.

Puncture from inside of pericarp disc indicated that all treatments generally had a similar curve in firmness loss (Figure 6.5D). Unlike other three measurement techniques, firmness loss in control fruit at constant 6 °C was not decoupled compared to the other treatments when plotted against colour change. It is possible that either this measurement technique may be a relatively insensitive method to detect differences in chilled and non-chilled tomato firmness at 6 °C or storage at 6 °C did not cause "severe damage" in tissue integrity in cell walls (revealed by inner puncture) rather it caused a loss in turgor primarily (seen in acoustic or compression of whole fruit) and loss of skin strength (seen in puncture of whole fruit from outside). Jackman et al. (1990) suggested that different firmness measurements may be more or less sensitive to severity of CI or extent of damage.

Overall, slope of firmness loss (measured by acoustic and compression) per unit change in colour was steeper for fruit stored at constant 6 °C than fruit exposed to IW. Fruit subjected to IW remained firmer than control fruit (6 °C) at a hue angle around 100° and subsequently loss of firmness in IW fruit after storage at 20 °C coordinating with red colour development. These results are in agreement with Artés et al. (1998b) who reported that IW maintained higher firmness than was seen in continuously chilled fruit at the end of storage and fruit were softened enough for immediate consumption at the end of post-chilling period at 20 °C. Application of 1-MCP in IW fruit retained higher firmness than IW alone; however, IW+1-MCP fruit delayed the co-ordination in ripening related softening suggesting a role of ethylene in softening of IW fruit. Importantly, when ethylene was applied intermittently at 6 °C-stored fruit, 6 °C+IE fruit showed a beneficial effect in retaining firmness during cool storage and restoring ripening (softening and colour change) on post-chilling removal to 20 °C.

6.3.1.4. Respiration rate

Respiration rate followed almost similar trend in all treatments (Figure 6.6). Overall 1-MCP treated fruit maintained a lower respiration rate than other treatments throughout 27 d at 6 °C or during warming period at 20 °C. In all three IW treatments fruit showed a surge in respiration rate during each warming period to 20 °C indicating an increased rate of metabolism. Since accumulation of toxic compounds caused by a temperature induced imbalance in metabolism was proposed as a possible cause of CI, the higher metabolic

activity in IW fruit may help tissues to remove excess intermediates or inhibited substances that accumulated during chilling and subsequently reduce CI.

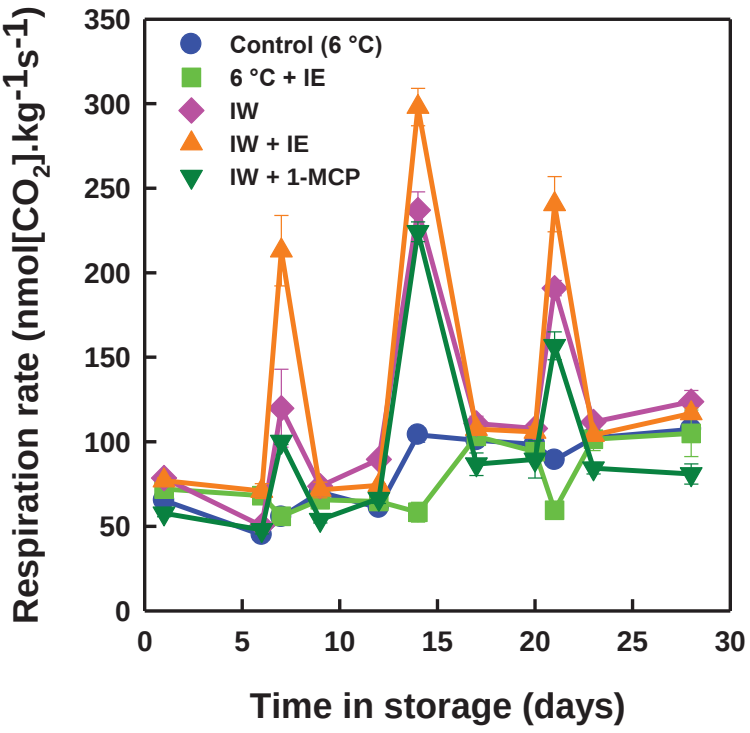

Figure 6.6 Postharvest respiration rate of mature-green tomatoes held under different treatment conditions for 27 d at 6 °C. Error bars represent the standard error (n = 15).

Higher respiration rate in IW+IE fruit measured at 20 °C compared to fruit subjected to IW (Table 6.3) suggested that exogenous ethylene influenced respiration (Kays and Paull, 2004). More importantly, when respiration rate was measured at cool storage (6 °C), IW+1-MCP fruit showed lower respiration rate than IW fruit (Table 6.3) indicating a role of ethylene influencing respiration. However, similar respiration rate (measured at 20 °C) between IW+1-MCP and IW alone fruit suggested that temperature was more influential compared with ethylene involvement in modifying fruit respiration rate. Additionally, exogenous ethylene at 6 °C (6 °C+IE) did not alter respiration rate in comparison to fruit maintained at 6 °C (Table 6.3), indicating that it was the physical effect of temperature of IW fruit which was more influential than ethylene itself in changing respiration rate. Normal red colouration of 6 °C+IE fruit after post-chilling storage at 20 °C indicates that

fruit tissues definitely perceived ethylene during cool storage, but that ethylene was insufficient to alter the respiration rate.

Table 6.3 Mean respiration rate (nmol.kg^{-1}s^{-1}) across all of the experiment duration (27 d) for mature-green tomatoes held at different treatment conditions. Respiration rate was measured at 6 °C for all treatments and at 20 °C for only those treatments that were subjected to IW.

Measurement temperature	Treatments	Mean respiration rate
6 °C	Control (6 °C)	81.28 bc
	6 °C+IE	84.33 ab
	IW	94.28 a
	IW+IE	92.18 a
	IW+1-MCP	72.21 c
20 °C	IW	182.60 b
	IW+IE	250.54 a
	IW+1-MCP	160.39 b

Mean separation within columns by LSD (α = 0.05).

6.3.1.5. Ethylene production

Ethylene production was the same in fruit from all treatments when measured on day 6 at 6 °C indicating that all fruit were at a similar physiological stage (Figure 6.7). After the 1st IW cycle, ethylene production was higher in IW fruit treated with or without exogenous ethylene than control fruit stored at constant 6 °C for the remaining storage period. No differences in ethylene production were recorded following ethylene application between control fruit and fruit at 6 °C treated with ethylene (6 °C+IE) ($p > 0.05$) for the 27-d storage period (Table 6.4). Adding external ethylene at low temperature did not lead to major stimulation of ethylene production; consistent with the idea that low temperature prevented metabolic production of ethylene in response to added ethylene.

Ethylene production was stimulated by each IW treatment when measured at 20 °C before dropping significantly when measured at 6 °C (Figure 6.7). Measurement of ethylene production was not possible for ethylene-treated fruit (6 °C+IE and IW+IE) during supply of 100 μL.L^{-1} exogenous ethylene. Therefore ethylene production for those ethylene-treated fruit was determined 2-d after each ethylene application to avoid any residual

effect. Exogenous ethylene for 24 h during each IW cycle (IW+IE) did not usually increase ethylene production when measured at 6 °C 2-d after ethylene application possibly indicating that IW fruit were already saturated with ethylene and at 6 °C, ethylene production rate was already maximised following IW. IW and IW+IE fruit reached ethylene climacteric peak before dropping ethylene production on day 27 (Figure 6.7).

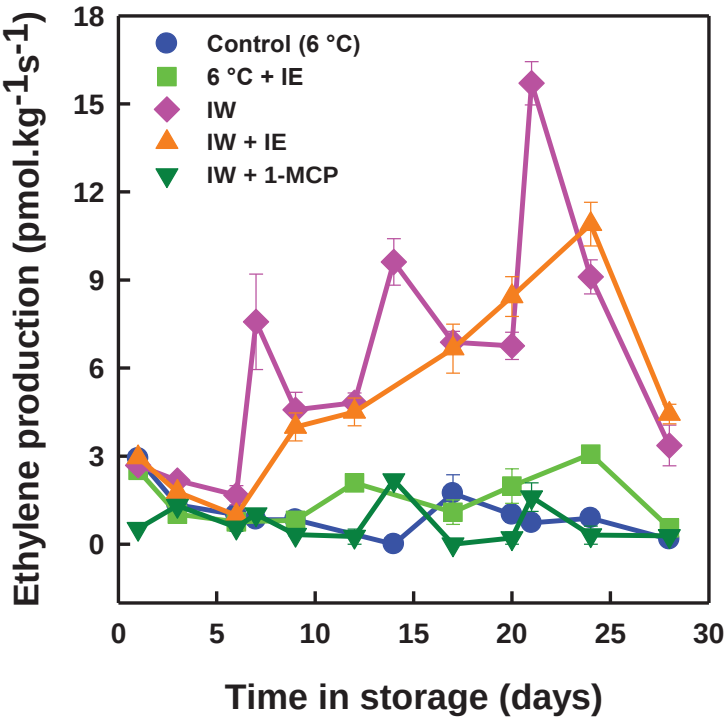

Figure 6.7 Ethylene production of mature-green tomatoes held under different treatment conditions for 27 d at 6 °C. Error bars represent the standard error (n = 15).

Ethylene production of fruit submitted to IW (3.36 pmol.kg^{-1}s^{-1}) was 20 times higher than fruit kept at constant 6 °C (0.17 pmol.kg^{-1}s^{-1}) at the end of 27 d storage (Figure 6.7). IW+1-MCP fruit produced overall lower ethylene than IW fruit. The IW+1-MCP fruit, however, showed a small increase in ethylene production during each warming period measured at 20 °C (Figure 6.7). However, this rise in ethylene production in IW+1-MCP fruit was significantly lower than fruit with IW alone ($p < 0.05$), indicating that 1-MCP simply inhibited autocatalytic ethylene production in IW fruit. More importantly, ethylene production of IW+1-MCP fruit returned to starting level when fruit were returned to 6 °C, suggesting simply warming stimulated ethylene production of fruit. Every warming cycle

caused fruit to accentuate ethylene production in IW fruit and advance in red colour development while IW+1-MCP did not allow fruit to increase overall ethylene production and fruit remained green (Table 6.4 and Figure 6.4).

Table 6.4 Mean ethylene production (pmol.kg^{-1}s^{-1}) across all of the experiment duration (27 d) for mature-green tomatoes held at different treatment conditions. Ethylene concentration was measured when fruit of all treatments were held at 6 °C.

Treatments	Mean ethylene production
Control (6 °C)	1.18 b
6 °C+IE	1.54 b
IW	4.66 a
IW+IE	4.95 a
IW+1-MCP	0.42 c

Mean separation within columns by LSD (α = 0.05).

Overall, storage at 6 °C for 27 d caused CI as indicated by blotchy red colouration, decay, and 'mild' texture damage (Figure 6.5). Fruit subjected to IW at 6 °C stimulated ethylene production and reduced CI and advanced ripening (red colour development, softening). Applying 1-MCP in IW treated fruit blocked autocatalytic ethylene response and inhibited red colour development and fruit softening during cool storage, indicating that ethylene is indeed required for IW to exert its benefit. 1-MCP fruit eventually initiated ripening after post-chilling period at 20 °C but fruit failed to attain full red colour rather were orange-yellow in colour indicating advancement of red colouration in IW fruit was ethylene dependent. However, blocking ethylene action by 1-MCP did not prevent all beneficial effects showed by IW. IW+1-MCP treatment reduced rot development suggesting that IW has some metabolic benefit independent of ethylene. Applying intermittent ethylene to fruit stored at 6 °C enhanced normal red colouration after storage at 20 °C and reduced CI, indicating a role of ethylene in reducing CI independently of increased temperature (IW) during storage. However, since fruit remained green in storage and attained normal red colouration only after post-chilling removal to higher temperature, it is possible both ethylene and higher metabolic activity is required for red colouration. Nonetheless, results indicate that tissue can perceive ethylene during cool storage but phenotypic changes only appear once fruit are transferred to warmer temperature. Therefore, it would be interesting if the same beneficial effect can be seen at 2.5 °C storage.

6.3.2. Part B – 2.5 °C storage

6.3.2.1. CI severity

In this section, results for tomatoes stored at 2.5 °C are reported. Tomatoes from all treatments stored for 13 d and subsequently removed to 20 °C, were a mixture of green or red fruit (Figure 6.8A). IW+IE treatment had highest percentage of red fruit (> 80%) followed by IW treatment (> 60%). No differences in CI symptoms were observed among treatments except that IW and IW+IE treatments both had reduced uneven blotchy red colouration significantly. Blocking ethylene response in IW fruit by 1-MCP increased blotchy red colouration and many fruit remained green suggesting advancement of red colour development in IW fruit was ethylene dependent (Table 6.5).

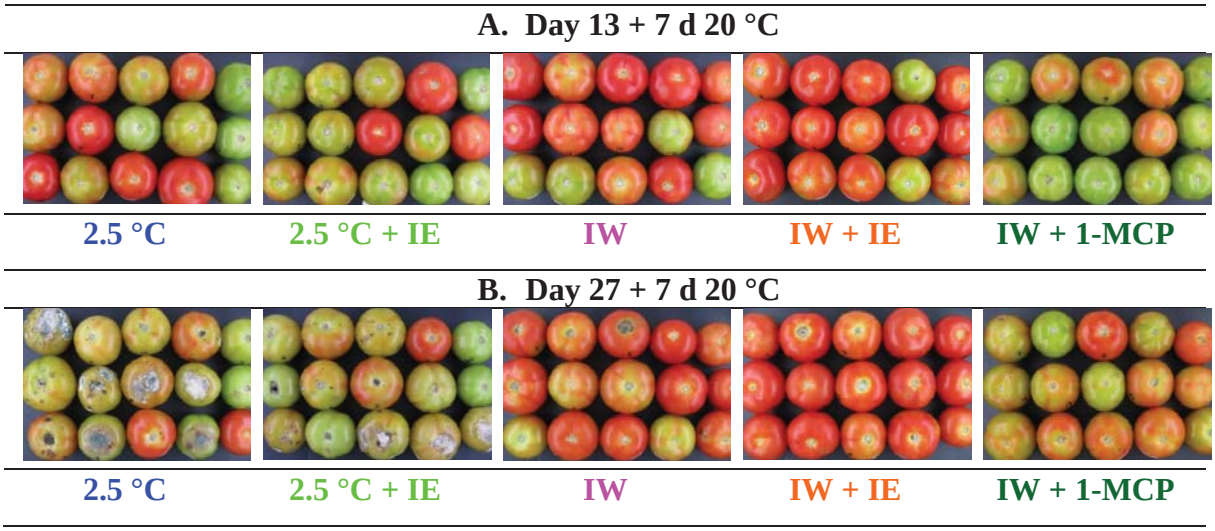

Figure 6.8 Visual appearance of mature-green tomatoes after 7 d post-chilling period at 20 °C following 13 (A) or 27 d (B) at 2.5 °C.

When time at 2.5 °C was extended to 27 d and fruit were evaluated at the end of post-chilling period at 20 °C, control fruit developed uneven blotchy colouration and showed severe decay (Table 6.6). Contaminating fungi were isolated on potato dextrose agar and incubated for 72 h at 25 °C; observations with a light microscope confirmed the presence of *Alternaria alternata*, *Botrytis cinerea* and *Penicillium* spp (Figure 6.8B). While different fungi cause tomato decay, *Alternaria alternata* is the organism that most often develops on the stem scar of tomatoes with chilling injury (Artés and Escriche, 1994). Unlike fruit maintained at 6 °C+IE, fruit from 2.5 °C+IE treatment did not develop red colour. IW treatment (with or without exogenous ethylene) advanced red colour development and

reduced decay significantly (Table 6.6). Compared with fruit subjected to IW at 2.5 °C described in chapter 4, in the present chapter IW fruit at 2.5 °C attained better red colour. Differences in growing season or variations in storage environments (e.g. higher RH) can explain these discrepancies (Barkai-Golan, 2001; Saltveit, 2005). Red colour development in fruit from the IW+1-MCP treatment was uneven and blotchy; suggesting advancement of red colour development in IW fruit involved ethylene response. However, like 6 °C storage, blocking ethylene action in IW fruit by 1-MCP did not prevent reduction of rot development indicating not all positive responses of IW were ethylene mediated. Additionally, like 6 °C storage experiment, IW+1-MCP fruit showed highest shrivel indicating some kind of toxic effect of high 1-MCP concentration (Table 6.6).

Table 6.5 Mean of CI severities (0 - 4 scale) of mature-green tomatoes after 7 d post-storage period at 20 °C preceded by 13 d under different temperature treatments (n=15).

Treatments	Shrivel	Rot	Blotchy colour
2.5 °C	0.07 a	0.13 a	2.27 b
2.5 °C + IE	0.00 a	0.13 a	2.53 b
IW	0.13 a	0.00 a	1.27 c
IW + IE	0.13 a	0.00 a	1.07 c
IW + 1-MCP	0.20 a	0.00 a	3.67 a

Mean separation within columns by LSD at $\alpha = 0.05$.

Table 6.6 Mean of CI severities (0 - 4 scale) of mature-green tomatoes after 7 d post-storage period at 20 °C preceded by 27 d under different temperature treatments (n=15).

Treatments	Shrivel	Rot	Blotchy colour
2.5 °C	0.47 c	2.20 a	3.33 a
2.5 °C + IE	0.53 bc	1.40 b	3.13 a
IW	1.20 b	0.13 c	1.27 b
IW + IE	1.07 bc	0.07 c	0.87 b
IW + 1-MCP	2.27 a	0.20 c	2.93 a

Mean separation within columns by LSD at $\alpha = 0.05$.

6.3.2.2. Surface colour

Fruit remained green when evaluated before removal to 20 °C and there were no differences among treatments after 27 d ($p > 0.05$; Figure 6.9). When fruit were transferred to 20 °C, IW fruit developed red colour (indicated by reduced hue) and this was accentuated when those IW fruit were treated with ethylene. Hue angle of fruit subjected to IW and IW+IE was 58° and 50° respectively at the end of post-storage period (Figure 6.9). Fruit from IW+1-MCP treatment did not develop full red colour (hue = 76°) instead colour was uneven and blotchy. A higher proportion of green fruit was found in fruit stored at constant 2.5 °C (hue = 81°) compared to fruit subjected to IW at the end of post-storage period at 20 °C. Importantly, exogenous ethylene applied to fruit at 2.5 °C did not advance the red colour development (hue = 86°). During the post-chilling period, hue angle in fruit stored at 2.5 °C with or without ethylene application reduced as compared to the value at harvest and uneven blotchy red colouration of some fruit contributed to this change in mean hue value.

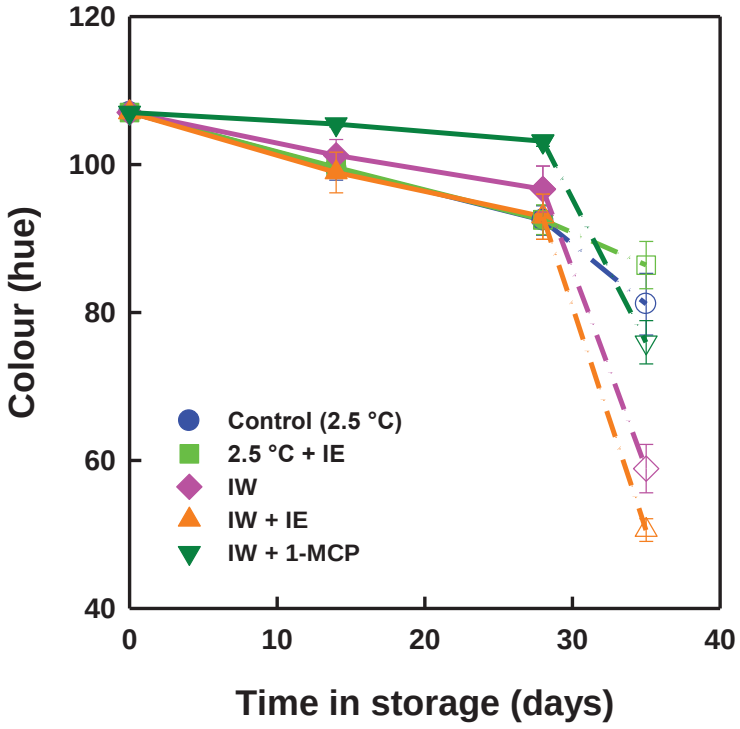

Figure 6.9 Surface colour of mature-green tomatoes held under different treatment conditions for 27 d at 2.5 °C. Dashed lines with open symbols indicate post-chilling transfer of fruit to 20 °C. Error bars represent the standard error (n = 15).

6.3.2.3. Fruit firmness

Firmness of tomatoes decreased progressively in all treatments (Figure 6.10). When fruit firmness was measured by acoustic sensor before removal of fruit to post-storage 20 °C, fruit from IW treatment had higher stiffness than control fruit at constant 2.5 °C ($p < 0.05$; Figure 6.10A). Once fruit were transferred to 20 °C following 27 d at 2.5 °C, both fruit at constant 2.5 °C and those subjected to IW softened but softening followed a different trajectory. IW coordinated softening with red colour development whereas storage at constant 2.5 °C (with or without ethylene) decoupled softening from colour change (Figure 6.10A). Therefore, since acoustic stiffness mainly measures tissue turgor (as discussed previously, section 2.3.3), turgor loss of control fruit at constant 2.5 °C was chilling-induced. Interruption of low temperature by periods of warming periods (IW) somehow restored the ripening process. When 1-MCP was applied in IW fruit, (IW+1-MCP) loss of stiffness in cool storage was inhibited. The 1-MCP treated fruit initiated softening and red colouration after removal to 20 °C; however, they failed to reach the same softening per unit change in colour as IW fruit without 1-MCP suggesting an ethylene response in softening of IW fruit. Fruit stored at constant 2.5 °C and treated with ethylene (2.5 °C+IE) did not advance red colouration, but retained higher stiffness compared to control fruit at the end of post-chilling period indicating a beneficial role of ethylene in retaining stiffness during cool storage. Similar effect was also observed during 6 °C storage experiment, although fruit maintained at 6 °C+IE also showed red colouration unlike fruit at 2.5 °C+IE.

Compression analysis also indicated the same trend as acoustic stiffness; however, the loss of firmness in fruit at constant 2.5 °C was less dramatic (Figure 6.10B).When fruit firmness was measured using an invasive puncture force from the outside of fruit epidermis, all treatments follow a similar path in changes in firmness (Figure 6.10C). However, when puncture force was applied from inside of a pericarp disc, differences in firmness loss between treatments were regained and the slope of softening curves of different treatments followed different paths (Figure 6.10D). Skin resistance possibly played a role in reducing differences between treatments when puncture was done from outside epidermis of whole fruit. When puncture force was used from outside, firmness values was twice that of puncture from inside of a disc. Like acoustic stiffness measurement, fruit stored at constant 2.5 °C softened without much colour change whereas IW fruit showed coordination of softening with simultaneous colour change on post-chilling removal to 20 °C, suggesting a chilling-induced softening in control fruit and in

contrast, softening in IW fruit was attributed to ripening. Since puncture force from inside of a pericarp disc largely determines tissue integrity, unlike 6 °C storage, accelerating firmness loss in fruit stored at constant 2.5 °C per unit change of colour compared to IW fruit could be credited to chilling-induced loss of tissue integrity (i.e. 'severe' chilling damage).

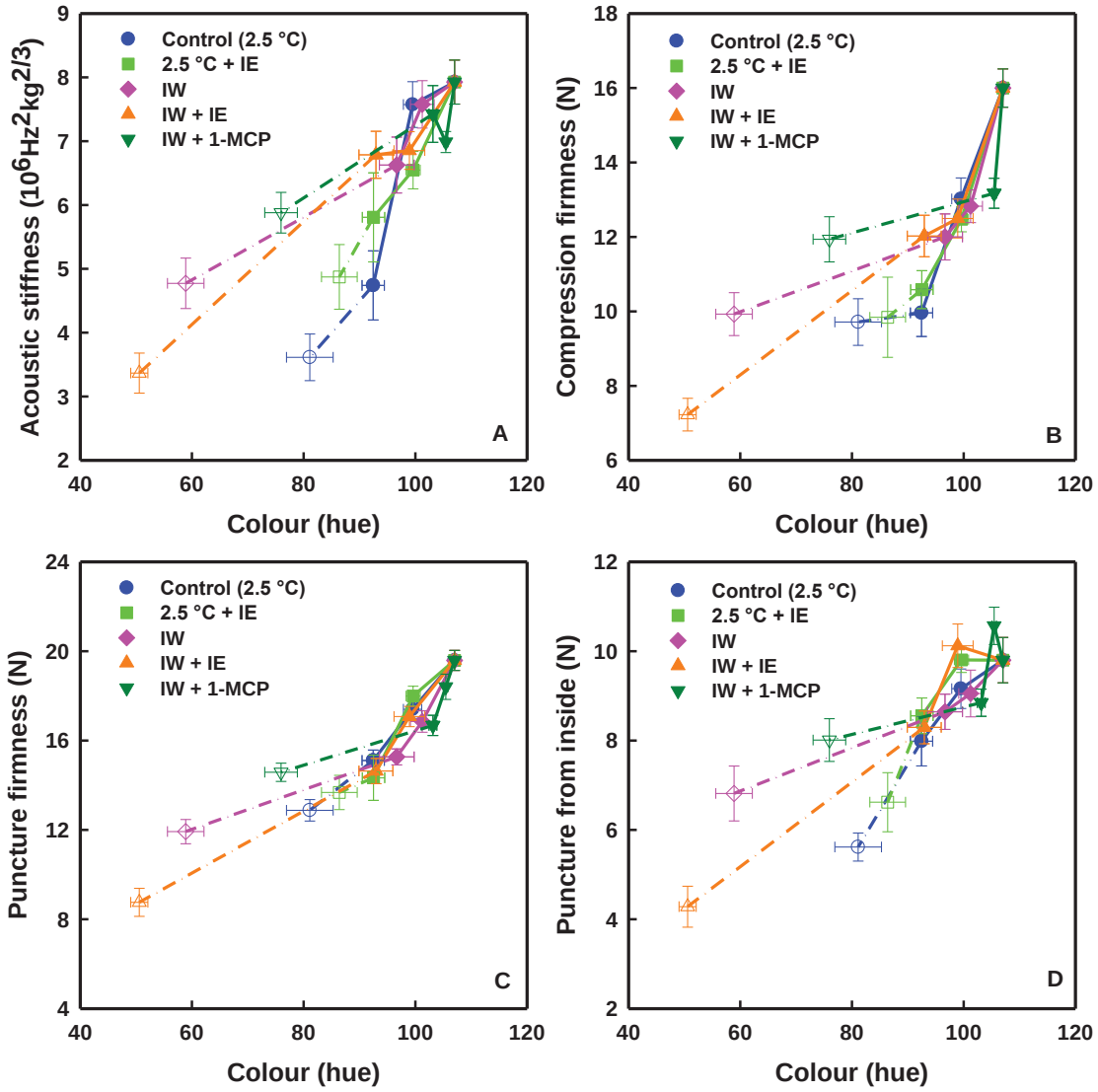

Figure 6.10 Relationship between colour (hue) and firmness of mature-green tomatoes held under different treatment conditions. Firmness was measured by acoustic sensor (A), compression force (B), puncture force (C) and puncture from inside of a pericarp disc (D). Dashed lines with open symbols indicate post-chilling transfer of fruit to 20 °C. Error bars represent the standard error (n = 15).

6.3.2.4. Respiration rate

Respiration rate increased significantly in all treatments from the value at harvest (Figure 6.11). When respiration rate was determined at cool storage (2.5 °C), no differences in respiration rate was observed between treatments except fruit from IW+1-MCP treatment (Table 6.7). A strong surge in respiration rate was observed when IW fruit were physically moved from 2.5 °C to 20 °C, indicating higher metabolism at the higher temperature. Fruit at IW+IE had the largest increase in respiration followed by IW alone. IW+1-MCP treatment maintained overall a lower respiration rate during each warming period than IW alone (Figure 6.11). Together these results indicate an involvement of ethylene in influencing respiration rate of IW fruit during warming period. Like 6 °C storage experiment, external ethylene could not significantly alter respiration rate in fruit stored at constant 2.5 °C. Previously, it was found in this chapter (6.3.1.4) and by others (Kays and Paull, 2004) that ethylene can alter respiration rate; however, temperature (metabolic activity) was more influential to alter respiration than ethylene itself.

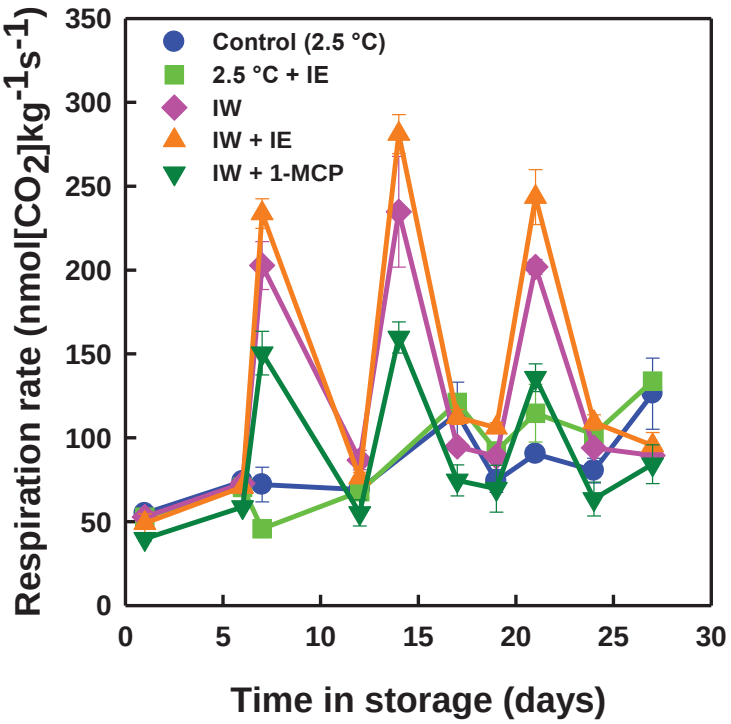

Figure 6.11 Postharvest respiration rate of mature-green tomatoes stored under different treatment conditions for 27 d at 2.5 °C. Error bars represent the standard error (n = 15).

Table 6.7 Mean respiration rate (nmol.kg^{-1}s^{-1}) across all of the experiment duration (27 d) for mature-green tomatoes held at different treatment conditions. Respiration rate was measured when fruit were maintained at 2.5 °C for all treatments and at 20 °C only for those treatments that were subjected to IW.

Measurement Temperature	Treatments	Mean respiration rate
2.5 °C	Control (2.5 °C)	84.82 a
	2.5 °C+IE	91.46 a
	IW	84.21 a
	IW+IE	90.42 a
	IW+1-MCP	63.68 b
20 °C	IW	209.44 b
	IW+IE	251.77 a
	IW+1-MCP	155.69 c

Mean separation within columns by LSD at $\alpha = 0.05$.

6.3.2.5. Ethylene production

Initial ethylene production (after 1 d at 2.5 °C) ranged from 1.4 – 2.3 pmol.kg^{-1}s^{-1} for all treatments except the IW+1-MCP fruit where it was 0.25 pmol.kg^{-1}s^{-1} and remained < 0.70 pmol.kg^{-1}s^{-1} through 17 d (Figure 6.12). No ethylene data was measured after 17 d at 2.5 °C due to equipment failure. During this 17 d period, IW fruit treated with ethylene (IW+IE) had the highest ethylene production rate compared to fruit in other treatments. After the 1st warming cycle, fruit subjected to IW showed higher ethylene production than control fruit kept at constant 2.5 °C but overall no differences in ethylene production between IW fruit and control fruit at 2.5 °C were observed for 17 d storage period. After one cycle of exogenous ethylene, 2.5 °C+IE treated fruit had lower ethylene production than fruit at 2.5 °C without ethylene as measured on day 12 before both treatment fruit had similar level of ethylene production on day 17 (Figure 6.12). On each warming occasion, there was an expected temperature-related jump in ethylene production in IW fruit and IW+1-MCP fruit.

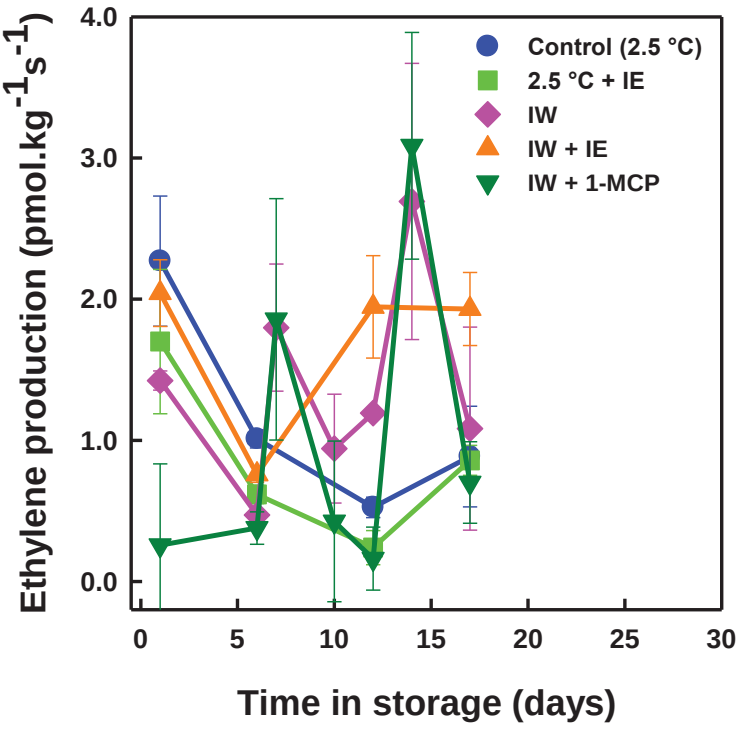

Figure 6.12 Ethylene production of mature-green tomatoes held at different treatment conditions for 17 d at 2.5 °C. Error bars represent the standard error (n =15).

As no ethylene data exists between 17 and 27 d storage, only assumption based on earlier work can be made. Irrespective of storage conditions, 'Cedrico' tomatoes reach their ethylene climacteric peak at a hue angle around 60° (Biswas et al., 2010). Similar observations were found in chapter 2 (Figure 2.3) and also reported for other tomato cultivars (Autio and Bramlage, 1986; Lurie and Klein, 1992). Therefore in this study, from the colour data, it can be assumed that fruit subjected to IW and IW+IE reached their climacteric peak as their hue angle were 58° and 50° respectively during post-storage period at 20 °C (Figure 6.9). Fruit in other three treatments (2.5 °C, 2.5 °C+IE and IW+1-MCP) did not develop red colour as indicated by hue (≈ 100°), therefore it can be assumed that those fruit did not reach their ethylene climacteric peak. Previously, non-detectable level of ethylene production (measured by GC) in 'Cedrico' tomatoes stored at 2.5 °C for 27 d was found during cool storage (Chapter 2, Figure 2.2).

Table 6.8 Mean ethylene production (pmol.kg^{-1}s^{-1}) across all of the experiment duration (27 d) for mature-green tomatoes held at different treatment conditions. Ethylene production was measured at 2.5 °C for all temperature treatments.

Treatments	Mean ethylene production
Control (2.5 °C)	1.17 ab
2.5 °C+IE	0.85 bc
IW	0.98 bc
IW+IE	1.67 a
IW+1-MCP	0.46 c

Mean separation within columns by LSD at α = 0.05.

Overall, storage at 2.5 °C for 27 d caused severe CI in tomatoes. Unlike 6 °C, fruit stored at 2.5 °C showed chilling induced 'severe' loss in firmness (i.e. perhaps loss in tissue integrity). IW at 2.5 °C reduced CI significantly (Table 6.5 and Table 6.6). IW fruit treated with 1-MCP showed an increase in blotchy colouration indicating red colour development in fruit maintained at IW was ethylene dependent. However, like 6 °C storage preventing ethylene response in IW fruit, IW+1-MCP also reduced rot suggesting not all beneficial effect of IW were ethylene-related. Ethylene supplied intermittently to 2.5 °C stored fruit (2.5 °C+IE) did not help fruit to develop red colouration but reduce decay incidence indicating beneficial effect of ethylene is storage temperature and nature of injury symptoms dependent. It appears that reduction of chilling-induced decay has both ethylene dependent and independent components.

6.4. Discussion

Failure to ripen, uneven blotchy red colouration and increased susceptibility to decay were major CI symptoms following exposure to low temperatures (Chapter 2). Additionally, different CI symptoms appeared to have different temperature threshold (Chapter 2). For instance, fruit stored at 8 °C showed delayed colour development without gross CI symptoms whereas fruit at 6 °C showed uneven blotchy red colouration and those at 2.5 °C were unable to develop red colour and showed severe decay.

Similar results were found in the present chapter where tomatoes stored at 6 °C for 27 d developed uneven blotchy red colouration whereas tomatoes stored at 2.5 °C failed to develop red colour upon post-chilling removal to 20 °C (Table 6.2 and Table 6.6). Three

cycles of IW during storage at 2.5 or 6 °C reduced CI symptoms, although IW at 2.5 °C storage was less effective than at 6 °C. The effectiveness of IW in reducing CI depends on storage temperature as previously demonstrated in chapter 4 and others (Artés and Escriche, 1994).

Chilling-induced loss of firmness was also previously reported as a CI symptom (Chapter 2). Both intermittent warming and continuous low temperature at 2.5 or 6 °C reduced fruit firmness progressively, however, with a significant different trajectory in firmness loss. Storage at constant 2.5 or 6 °C decoupled softening from colour change (i.e. uncoordinated ripening) whereas IW restored the coordination of fruit softening with ripening (Figure 6.5 and Figure 6.10). This implies that softening in fruit stored at 2.5 or 6 °C was chilling-induced whereas for the fruit subjected to IW was ripening-related, consistent with earlier results (Chapter 2). Use of different measurement methods provided insight into modes of CI and allowed speculation about impact of CI at different storage temperatures. For instance, measurement of texture change by puncture force from inside a pericarp disc of 6 °C-stored fruit failed to differentiate the softening trajectory whereas the same method showed a deviation for fruit stored at 2.5 °C. It is possible that storage at 6 °C caused only 'minor' changes in texture such as loss of turgor (usually detected by acoustic stiffness and compression firmness) whereas storage at 2.5 °C resulted in 'severe' chilling damage and loss of tissue integrity (shown by dramatic change in internal puncture resistance).

Reduction of CI by IW could be associated with advancement of normal ripening as it was reported for peaches and nectarines (Dong et al., 2001; Zhou et al., 2001). In our experiment at 6 °C, IW stimulated higher ethylene production than fruit at constant 6 °C and advanced fruit ripening indicated by red colour development accompanied with fruit softening (Figure 6.4 and Figure 6.7). Therefore, it can be argued that intermittent warming during low temperature storage is advancing ripening and inducing chilling tolerance because chilling sensitivity reduces as tomatoes ripen (Autio and Bramlage, 1986). When IW fruit were treated with 1-MCP to block IW-stimulated ethylene response, fruit failed to develop full red colour and remained green in storage (Figure 6.4 and Figure 6.9). Although 1-MCP treated fruit eventually resumed ripening as indicated by softening with simultaneous colour change after 7-10 d at 20 °C, these fruit failed to achieve the same colour and ripening-related softening as fruit without 1-MCP. 1-MCP-treated IW fruit from 6 °C showed yellow-orange colouration and those from 2.5 °C had an uneven blotchy

colouration during post-chilling period at 20 °C. It appears that there were some disturbances to the biochemical changes associated with normal ripening (Golding et al., 1998; Mostofi et al., 2003). Since alteration in normal red colouration during low temperature storage is the main CI symptom, these results indicate that a certain level of stimulated ethylene production during IW was necessary during low temperature storage to obtain a beneficial effect of red colour development. Added ethylene during warming (IW+IE) resulted in more advanced ripening with fruit being redder and softer than IW tomatoes without ethylene, specifically in fruit stored at 2.5 °C, consistent with the idea of involvement of IW-stimulated ethylene in advancing red colour development. However, IW+IE treatment in fruit stored at 6 °C did not show any added benefit (in terms of red colour development) compared to IW fruit without ethylene. Perhaps IW fruit at 6 °C was already saturated with ethylene. Saltveit (1999) indicated that once climacteric fruit initiated ripening, internal ethylene concentration quickly rose to saturation level and exogenous ethylene no longer accelerated ripening.

Increased susceptibility to decay is a CI symptom. Fruit stored at constant 2.5 or 6 °C developed decay during their post-chilling period at 20 °C. At 6 °C, tomatoes exposed to IW showed stimulated ethylene production and reduced decay. Fruit subjected to IW during 2.5 °C storage had reduced decay largely in the absence of stimulated ethylene production. More importantly, blocking ethylene response of IW fruit by 1-MCP also reduced decay suggesting that IW possibly has some metabolic benefit independent of ethylene.

This idea is consistent with our earlier experiments where no differences in ethylene production were found in fruit stored continuously at 2.5 °C or intermittently warmed during 27 d (Chapter 4, Figure 4.3), yet IW reduced decay during the post-chilling period. Since IW reduces chilling-induced decay irrespective of whether fruit produces ethylene or not, this warrants discussion in respect of some other mechanisms. It is possible that warming the fruit intermittently during cool storage maintains the physical barrier of cuticle and suberin, whereas storing fruit in too low temperature for a long time could prevent the formation of suberin by the cells surrounding the picking wound and ultimately facilitate pathogen attack (Barkai-Golan, 2001). It is also possible that IW activates defence mechanisms by inducing resistance or temporarily warming may allow cool stored

tomatoes to maintain preformed inhibitors and induce phytoalexins, as seen during other heat treatments (Fallik et al., 1996; Lurie et al., 1997).

As adoption of IW in commercial situation is logistically challenging, one objective of the current experiment was to determine if intermittent ethylene supply during low temperature storage could be used as an alternative to IW for alleviating CI in tomato. Tomatoes stored at 6 °C for 27 d and treated with three cycles of intermittent ethylene (IE) developed normal red colour and showed reduced decay at post-chilling transfer to 20 °C, indicating a possibility of using IE as an exciting strategy to reduce CI. However, fruit stored under the same IE treatment at 2.5 °C were unable to develop red colouration after storage at 20 °C although they showed reduced decay, indicating effectiveness of IE was temperature dependent. Inconsistent results of ethylene response in reducing CI in tomato have been reported. While Chomchalow et al. (2002) found that tomatoes treated with ethylene prior to storage at 2.5 °C showed better colouration (orange-red) than tomatoes without ethylene (colour development was inhibited), others reported that exposing mature-green tomatoes to ethylene before or after storage at chilling temperatures below 5 °C did not affect CI symptoms (Kader and Morris, 1975; Ogura et al., 1976).

Fruit stored at 6 °C and treated with intermittent ethylene (6 °C+IE) remained green during 27 d cool storage but developed normal red colour at the end of post-chilling period at 20 °C. This indicates that tissues at 6 °C must have perceived ethylene during cool storage but the associated phenotypic changes (e.g. red colouration) only appeared once fruit were moved to ambient temperature after storage. It is possible that ethylene was perceived by the ethylene receptors and a signal was transduced to the chloroplast to initiate the conversion to chromoplast. However, since enzyme kinetic properties in plant is influenced by chilling temperature (Caldwell, 1990), it is possible that enzymes related to biochemical reaction (lycopene synthesis or chlorophyll degradation) were not activated yet due to presence of chilling temperature and hence, fruit remained green. It seems that solely the presence of ethylene is not enough with sufficient metabolic activity of the tissue being essential to develop red colouration in tomato. On the other hand, higher metabolic activity without ethylene response, in case of IW+1-MCP fruit, did not lead to development of red colouration, but IW without 1-MCP did advance red colouration during cool storage. These data suggest that both ethylene and a sufficient metabolic activity (enzyme activity) are required for red colour development in tomato.

For fruit stored at 2.5 °C, it is possible that there was not enough ethylene perceived by the tissue for normal red colouration but enough for decay reduction. Ethylene binding capacity can be decreased during low temperature storage (Jiang et al., 2004), but ethylene perception was by no means completely inhibited for those fruit at 2.5 °C. Therefore, it is possible that there was sufficient ethylene in tissue to transduce the signal to chloroplasts but the chloroplast-chromoplast conversion was severely damaged during cool storage and thus, fruit failed to develop normal red colouration. Chomchalow et al. (2002) attributed blotchy red colouration of tomatoes maintained at 2.5 °C and pre-storage treated with ethylene to damage of chloroplast-chromoplast conversion.

Ethylene has an ambiguous effect on decay development (Porat et al., 1999). In the current study, exogenous ethylene application reduced decay susceptibility in fruit stored at 6 or 2.5 °C (Figure 6.2 and Figure 6.6). Additionally, treating tomatoes stored at 2.5 °C with 1-MCP enhanced chilling sensitivity as indicated by increased decay susceptibility (Chapter 5), consistent with ethylene involvement in reducing decay during cool storage. Jing and Zi-Sheng (2011) also indicated that treatment of green tomatoes with 1-MCP enhanced decay susceptibility in fruit stored at 3 °C. On the other hand, IW reduces decay without influencing ethylene production or ethylene responses. It seems that mechanisms of decay reduction in tomato were both ethylene dependent and independent.

The susceptibility of fruit and vegetables to decay increases as ripening progresses. Ethylene promotes ripening and advances fruit senescence and hence, ethylene can increase decay susceptibility via advancement of ripening. That may explain why other researchers report a reduction of decay in tomatoes by 1-MCP. For instance, 1-MCP treatment reduced decay of mature-green tomatoes stored at 18 °C (Su and Gubler, 2012), possibly by keeping the fruit in more resistant unripe condition. In contrast, 1-MCP increased decay for cool-stored tomatoes in this study (Chapter 5) and in that of Jing and Zi-Sheng (2011). Since chilling sensitivity is higher for green tomatoes than ripe tomatoes, it is possible that 1-MCP prevents the fruit advancing in ripening and renders fruit more susceptible to chilling. Importantly, since ethylene showed beneficial effects in reducing chilling-induced decay, it is logical to suggest that 1-MCP may have interfered with ethylene-induced resistance in ripening tomato fruit. Overall, results suggest that ethylene has a differential role in influencing decay and that may explain, at least in part, the inconsistent reports about effectiveness of ethylene in reducing decay.

Another interesting observations needs to be clarified. During each intermittent warming occasion, 1-MCP treated fruit showed stimulated ethylene production (Figure 6.7 and Figure 6.12). Stimulated ethylene production of 1-MCP treated fruit could be due to loss of negative feedback regulation of ethylene biosynthesis (Watkins, 2006). However, the ethylene production dropped to starting levels once fruit were returned to cool storage, suggesting that this increase in ethylene production during IW was solely a response of temperature. Treatment with 1-MCP typically suppressed ethylene production (Blankenship and Dole, 2003); however, it enhances ethylene production in mature-green bananas (Golding et al., 1998) or avocados (Jeong et al., 2003). Moreover, 1-MCP stimulated ethylene biosynthesis in non-climacteric mandarins (Salvador et al., 2006), grapefruit (McCollum and Maul, 2007), and leaves of coriander (Jiang et al., 2002a). In some cases increased ethylene does not appear to affect other senescence process e.g. 1-MCP treated citrus fruit remained green despite higher ethylene production (Mullins et al., 2000; McCollum and Maul, 2007). Salvador et al. (2006) attributed the 1-MCP induced ethylene production to be a defence mechanism against CI development in mandarins. In the present study, the effect of 1-MCP stimulated ethylene production during IW was unclear. Tomatoes remained green in the IW+1-MCP treatment despite a surge in ethylene production during each warming occasion. On the other hand, IW without 1-MCP stimulated ethylene and induced fruit to advance to the climacteric peak and red colour development particularly for the fruit stored at 6 °C. These findings suggest that not only is IW stimulated-ethylene production essential to induce chilling tolerance but synthesis of new ethylene receptors and capacity of those receptors to bind ethylene are also important. Since ethylene receptors were blocked by 1-MCP, IW+1-MCP treatment did not induce fruit to advance to the climacteric peak nor to develop red colour when fruit were still in storage despite the surge in ethylene production during the warming period. Lack of red colour development probably indicates that receptors were not regenerated. 1-MCP treated tomatoes, however, turned yellow-orange or blotchy coloured upon post-storage transfer to 20 °C. It is possible that IW+1-MCP-treated tomatoes were able to make new ethylene receptors and ultimately overcame the inhibition of 1-MCP (Sisler et al., 1996). In 1-MCP treated bananas, it has been suggested that heat treatment enhances synthesis of new ethylene receptors (Jiang et al., 2002b).

6.5. Conclusion

IW alleviated CI in tomato; however, effectiveness was dependent on storage temperature and CI symptom. Fruit stored at constant 6 °C produced negligible ethylene during cool storage and showed uneven blotchy red colouration and became susceptible to CI. The reduced level of ethylene production of chilled fruit may be responsible for the failure of these fruit to ripen normally. On the other hand, the increased ethylene production of IW fruit assisted development of full red colour which coincided with softening. These results suggest that IW somehow enables fruit tissue to retain the capability to ripen normally. This advanced fruit maturity may contribute to induction of chilling tolerance (Autio and Bramlage, 1986). Zhou et al. (2001) suggested that IW reduces CI of peaches by enhancing ethylene production and enzymes mediated by ethylene.

Additionally, supplying ethylene during IW could add increased benefit depending on storage temperature. Intermittent supply of ethylene during low temperature storage allowed tomatoes to ripen with subsequent alleviation of CI. Although ethylene application can reduce CI in many crops including tomato, to our knowledge this is the first report where an intermittent ethylene supply during cool storage succeeded in reducing tomato CI.

When IW-stimulated ethylene action was blocked by 1-MCP, fruit remained green during 27 d storage. Upon post-storage transfer to 20 °C for 7-10 d, those fruit developed an orange-yellow or blotchy uneven colouration. This indicates that blocking the action of IW-stimulated ethylene by 1-MCP rendered the fruit susceptible to low temperature stress. Previously, it was demonstrated that 1-MCP treated fruit stored at 2.5 °C tended to have a greater inhibition of colour development, chilling-induced softening and higher decay severity than non-treated control (Chapter 5). Collectively, these data suggest that stimulation of ethylene production by IW is essential to reduce CI in tomato. However, reduction of CI symptoms by IW could be ethylene independent based on the nature of the symptoms. Reduction of chilling induced decay in IW fruit treated with 1-MCP indicated that the mechanism for chilling injury protection by IW may not be solely attributable to ethylene action.

An inter-relationship between ethylene production and CI has been proposed (Wang, 1989). Application of exogenous ethylene before low temperature storage to alleviate CI in

tomato was documented. Post-storage ethylene application was not as beneficial as pre-storage (Chomchalow et al., 2002). It was suggested that intermittent ethylene supply during 'mild' low temperature storage could potentially reduce CI in tomato. In this experiment, IE at 6 °C for 4 weeks resulted in 100% marketable fruit (10 d after removal from chilling temperature), whereas without IE, less than 30% fruit were marketable. Since both intermittent warming and intermittent ethylene supply can reduce CI in tomato and determining the optimum conditions for IW storage that will favour commercial adoption is physically challenging, these results indicate that intermittent ethylene supply during cool storage can be used as an alternative approach to IW for reducing CI in tomato. Indeed intermittent ethylene supply is easier to apply than intermittent warming in commercial situation. While IW involves repeated increase and decrease of storage temperature, that is usually a slow and lengthy process or moving the fruit to cool storage to higher temperature, that is labour intensive; gas treatment is definitely easy and quick to achieve in a large scale commercial coolstore. Overall, the positive results of ethylene application may enable the tomato industry to store tomatoes for longer periods at chilling-inducing temperatures and hence enable sea freight of tomatoes to new markets. However, effectiveness of ethylene supply is a function of storage temperature as reported in case of IW. Ethylene application dose and timing would be critical factors too to exploit it's (i.e. IE as a technique) full potential. Therefore, optimisation of storage temperature to ethylene treatment and ethylene concentrations is something that we would be looking to investigate in future research. While the findings are positive for a possible industry application, the magnitude of the positive effect in reducing CI needs to be determined for other tomato cultivars and other growing locations. Additionally, if the recommendation of IE application is commercially achieved, in future it is important to investigate the effect of IE in sensory perception of tomato quality.

7. Overall discussion and future studies

7.1. Introduction

Tomato is an important crop both commercially and nutritionally. Commercially, tomatoes are harvested at the mature-green stage and handled at low temperature to facilitate shipping (Chomchalow et al., 2002). However, long term low temperature storage of mature-green tomatoes is challenging because of the likely development of CI. In New Zealand, export of tomatoes is limited to expensive air freight. Apart from other marketing reasons, industry still does not have a consistent commercial solution for exporting mature-green tomatoes to distant markets by sea. It may be possible to export New Zealand-grown tomatoes by sea to emerging Asian markets if a potential solution to enable successful long term storage is found. Importantly, if tomatoes can be supplied for an extended period, this may help handlers of fresh tomatoes to reduce gluts in the market and to supply products at times of shortage. Ultimately, it may provide growers a better return.

There is a general consensus that cell membrane damage under low temperature stress is the primary cause of CI (Lyons, 1973). Sustained primary damage then leads to a series of secondary reactions, resulting in oxidative stress, metabolic dysfunction, and irreversible manifestation of visible symptoms. Early detection and diagnosis of CI is difficult, as the injured fruit often looks sound as long as it remains in low temperatures. Symptoms generally appear when chilled produce is placed in warmer temperatures. A substantial portion of losses resulting from CI may be mistakenly credited to pathogen-induced or ripening disorders in the market (Wang, 1994).

The ultimate goal of CI studies is to alleviate this disorder. Many techniques have been suggested for reducing CI. One of them is intermittent warming. Given the nature of CI mechanisms in general, it appears that occasional interruption of low temperature period with a periodic warming period (IW) prevents CI damage.

Despite positive results, IW is rarely practised commercially because of practical difficulties associated with periodically warming large volumes of fresh produce. Repeatedly increasing and decreasing storage temperature is usually a slow and lengthy process (depending on packaging) that is expensive to achieve quickly (e.g. by forced draft heating or cooling) because it is energy intensive. In addition, condensation problems

during warming and cooling cycles may lead to fungal and bacterial rots. The optimum regime may differ among cultivars, with fruit maturity stage and growing conditions. Generally, a successful IW regime is arrived at empirically, by trial and error, and what works on one cultivar or growing condition is not as successful on another. It is potentially more valuable to investigate basic mechanisms of how IW reduces CI instead of trying to optimise another successful regime for a species or cultivar. A deeper understanding of the mechanisms by which IW exerts its beneficial effects may allow us to identify novel techniques that will harness the benefits of IW without the logistical problems that are associated with industrial application. Additionally, this understanding may help to suggest a novel technique which is more practical and may be quite different from IW. If the technique is commercially viable and applicable, it could aid the tomato industry, in New Zealand and elsewhere, to export fresh fruit to new markets. In addition, it may help to extend the tomato supply window and reduce market gluts in the local market. From a scientific point of view, an understanding of the influence of IW on CI could provide information about the physiological and biochemical basis of the disorder.

7.2. Defined CI symptoms in tomato and their time-temperature threshold

'Threshold temperature' is defined as the lowest temperature at which a susceptible fruit or vegetable can be stored with no symptoms of CI ever developing (Brecht et al., 2012). A storage temperature considered safe for mature-green tomatoes is reported as 13 °C (Hobson, 1981). Below this 'threshold temperature', the current study indicated that different low temperature ranges affected tomatoes differentially as characterised by different CI symptoms. For a given storage duration (e.g. 27 d), storage of fruit at 8 °C delayed (but did not prevent) red colouration, fruit maintained at 6 °C showed blotchy red colouration with occasional decay and those at 2.5 °C showed a complete failure of normal colouration and severe decay (Chapter 2). Moreover, analysis of data by using a range of textural methods indicated that storage at 6 °C mainly induced loss of turgor whereas 2.5 °C induced loss of tissue integrity along with turgor loss (Chapter 6). Overall, it appears that there is a series of critical temperature thresholds at which different CI symptoms are induced in mature-green tomato fruit.

The response of plant tissues to chilling injury has frequently been separated into primary and secondary events. The sustained damage that occurs as a result of the primary events causes a cascade of secondary effects that are reflected as injury symptoms. However,

there does not seem to be any obvious reason to suppose that all secondary events happen simultaneously in a chilling damaged tomato tissue. It is possible that a temperature sequence occurs where there is a higher temperature threshold for some symptoms than others; or that is, there is a time sequence in which the symptoms will appear at a given temperature. Suggesting a 'threshold temperature' for a particular CI symptom is, therefore, oversimplified. Each symptom observed after storage at a particular temperature for a certain period may be triggered by a lower chilling temperature in a shorter period of time. Generally, the severity of injury of sensitive tissues increases as temperature is lowered or as exposure is extended at any chilling temperature (Saltveit and Morris, 1990).

A sequence of time-dependent events at a given temperature is reported. Cheng and Shewfelt (1988) indicated that tomatoes stored at 4 °C and subsequently removed to 21 °C showed an increased decay after 15 d, and after 27 d ethylene production started to decline (perhaps chilling damaged the system which converts ACC to ethylene), and after 34 d retardation of colour development began to appear. A post chilling evolution of CO_2 production still occurred in fruit stored as long as 39 d. They suggested that plasma membrane, vacuoles, chloroplasts and mitochondria were in decreasing order of susceptibility to chilling temperature. Similarly, an ultrastructural study of tomato by Moline (1976) revealed that at a given temperature (2 °C), chilling of fruit for 10 d interfered with conversion of chloroplasts to chromoplasts. After 15 d, mitochondria and plastids swelled and degenerated and after 21 d, organelles were barely visible. In the present study, fruit stored at 2.5 °C showed an increased electrolyte leakage after 2 weeks (Chapter 3) and severe decay by 4 weeks (Chapter 2). Red colour development was altered after 2 weeks and complete failure to red colouration by 4 weeks. These results suggest that at a given chilling temperature there is a sequence of chilling-induced damage incurred by different organelles.

Metabolic activity of the fruit is directly but differentially influenced by low temperature also. Kinetic parameters of enzymatic reactions are known to be temperature dependent and low temperature can affect protein and enzyme functions directly (Graham and Patterson, 1982). Therefore, it is possible that at a given chilling temperature enzyme activities related to different processes that lead to injury symptoms will be affected differently. For example, it is possible that activation energy of enzymes associated with

lycopene synthesis or natural defence mechanisms are different and that resulted in a differential extent of damage at different chilling temperature.

Since at a given time, different chilling temperature may result in different symptoms or at a given temperature, different processes (organelles structure, enzyme activity or protein functions) may be hampered sequentially, it is possible that each symptom has an independent time-temperature threshold to appear and this may explain why some symptoms appear earlier than others.

By examining the literature on different CI symptoms of mature-green tomatoes, a conceptual model is created to determine if different chilling thresholds as influenced by temperature and time of exposure exist for different chilling symptoms (Figure 7.1). The observed CI symptoms are divided into seven categories - flavour loss, blotchy red colouration, complete failure to ripen, excessive softening, pitting/sunken patches, decay, and ion leakage. First the appearance of symptoms described in each study was plotted on a time-temperature axis. Each symptom was grouped by identifying a line of the first time of appearance of a symptom at a temperature indicating the potential sensitivity of each symptom to chilling. Across the published literature, tomatoes differ in genotype and growing location, while this can introduce error, it does not necessarily prevent identification of any generic effects that may apply across cultivars and growing regions. This conceptual model is, however, limited since recording of the symptoms occurs after a 'pre-decided' chilling duration, and many studies did not involve earlier examination of fruit. This model presents conditions that are known to cause symptoms, as opposed to a conservative model, which would identify regions where there would be a risk of symptoms occurring.

Analysing the resulting model (Figure 7.1), it seems that there are four different trends (as shaded) for chilling symptoms. Some symptoms appear very rapidly, some are rapid but take a little longer than others, while some are intermediary, and some are slow to become evident.

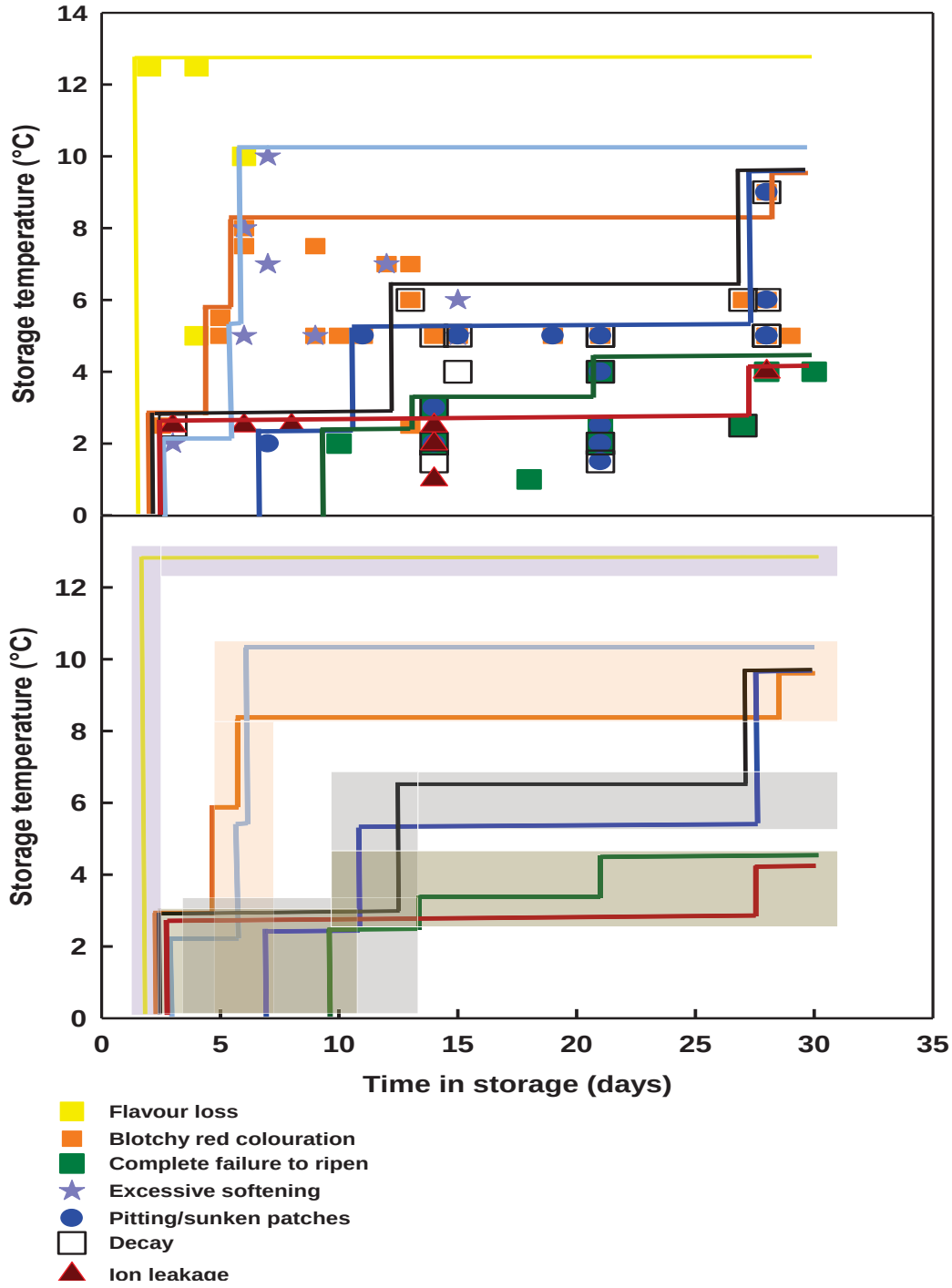

Figure 7.1 Conceptual model for sensitivity range for different CI symptoms observed by researchers at a given time-temperature regime. **(Source: Data were retrieved from Artés and Escriche, 1994; Bergevin et al., 1993; Cheng and Shewfelt, 1988; Chomchalow et al., 2002; Diáz de León Sánchez et al., 2009; Ding et al., 2001, 2002; Efiuvwevwere and Throne, 1988; Hobson, 1981; Jackman et al., 1990; Kader et al., 1978; Luengwilai and Beckles, 2010; Luengwilai et al., 2012; Lurie et al., 1997; Lurie and Klein, 1991, 1992; Lurie and Sabehat, 1997; Malacrida et al., 2006; Marangoni et al., 1995; Maul et al., 2002; McDonald et al., 1999; Sabehat et al, 1995; Saltveit, 2002, 2005; Sanchez-Bel et al., 2012; Soto-Zamora et al., 2005; Vega-Garcia et al., 2010).**

Starting with the fastest-appearing problem, a storage temperature above what is recommended for mature-green tomatoes (13 °C) has an adverse effect on flavour even when tomatoes are stored for as little as 2-4 d. Relatively rapid appearing injury symptoms are blotchy red colouration and excessive softening. Blotchy red colouration was reported to occur after a week at 7-8 °C. However, blotchy red colouration was reported after only 3 d at 3 °C. Similarly excessive softening was found after 3 d at 2.5 °C but only after 7-8 d at 10 °C.

Pitting or sunken patches followed by decay seems to have intermediary sensitivity. Pitting is not generally found until after 7-8 d even at very low temperatures such as 2.5 °C. If storage temperature is a little higher (5 °C), it takes approx 11 d to develop pitting and 28 d at 9 °C. Pitted tissue is often an ideal entry point for microorganisms, especially fungal saprophytes. Although an increase in susceptibility to decay is a complex mechanism because of multifaceted host-pathogen interactions (Barkai-Golan, 2001), previous report indicates that pitting is usually followed by decay incidence. Overall, results suggest that pitting and decay may require slightly longer time to express than altered colour development.

An example of a very slowly developing CI symptom is complete inhibition of red colouration (failure to ripen). This is mainly reported at a temperature after 28 d at ≤ 5 °C. Even around 3 °C, this symptom takes a relatively longer period (around 9 d) than the exposure duration required for the blotchy red colouration (3-4 d). It appears that susceptibility to decay was more sensitive to chilling temperature than complete inhibition of red colouration/failure to ripen (Cheng and Shewfelt, 1988; Artés and Escriche, 1994). An increased rate of ion leakage has not been reported at temperature above the range 1-4 °C and thus may require extreme chilling to develop. It is of course possible that researchers have not looked for increased ion leakage at relatively warmer temperature. However, in our ion leakage studies, tomatoes stored at 6 °C for 7 d did not show an increase in ion leakage (section 3.3.1). Even at 2.5 °C, significant increase in leakage was not noticed until after around 14 d suggesting the slow development of this symptom.

Overall, there is a ‘threshold temperature’ for storing mature-green tomatoes below which fruit start to show a series of chilling-induced symptoms. Below this threshold temperature, time and temperature together set the thresholds for onset of damage and different

symptoms have a different time and temperature threshold. There is a temperature sequence where some symptoms (flavour loss, blotchy red colouration) are triggered at higher temperature than others (complete inhibition of red colouration, pitting or decay). Thus below 13 °C, the recommended storage temperature at a given time, mature-green fruit will first lose their flavour, then show blotchy red colouration and altered texture, then develop pitting accompanied by increased susceptibility to decay (mainly *Alternaria*) and if it is too long in that chilling temperature, fruit will fail to ripen completely.

While each symptom may have a time-temperature threshold, prior growing condition, cultivar, stage of development or storage environment (e.g. RH, gas composition in store-room atmosphere) may shift this time-temperature threshold. For example, in this work, storage at 6 °C for 27 d did not induce decay for New Zealand-grown 'Cedrico' tomatoes (Chapter 2), with the exception of one experiment. Differences in growing season or variations in storage environments (e.g. higher RH) can explain these discrepancies (Barkai-Golan, 2001; Saltveit, 2005). Decay was consistently found in tomatoes ('Soraya') grown in Florida after storage at both 2.5 and 6 °C for 13 or 27 d (Chapter 2). This suggests that chilling sensitivity was enhanced for 'Soraya' tomatoes and for them a chilling symptom (e.g. decay) started to develop at a relatively higher temperature and a shorter time (i.e. chilling threshold bar has increased) than what was found in 'Cedrico' tomatoes. Patterson and Reid (1990) indicated that threshold temperature is often typical for the species, although it is not always a good indicator of overall sensitivity to chilling.

While those factors (cultivar, pre-harvest growing condition, stage of development or RH) can influence time-temperature threshold bar for a symptom, is it possible that they can alter sensitivity of each symptom differentially, causing a different sequence of symptoms to appear? In Florida, only 2.5 and 6 °C were investigated as chilling temperatures and both temperatures showed blotchy red colouration and severe decay. Therefore the potential region in which the time-temperature combination results in blotchy red colouration, but not the decay, was not found. Perhaps a temperature around 9-10 °C is that potential region, since slower red colour development was seen in fruit stored at 12.5 °C compared to 20 °C (Figure 2.1B). Therefore at a given time, blotchy red colouration will appear first, then decay incidence. Overall, it is possible that external pre and post-harvest factors can shift the time-temperature threshold for a symptoms but not the sequence of secondary responses to appear.

Although initiation of secondary events may appear sequentially and different low temperatures induce different CI symptoms, it is not obvious whether this is a result of different time-temperature threshold for damage or just that the phenotype takes longer to display. For example, underlying damage for the initiation of blotchy red colouration may occur in a particular time-temperature threshold level, but the phenotype can only be seen after certain time. Until colour begins to appear during ripening it is impossible to detect unevenness in that colour. Chomchalow et al. (2002) noticed that there was an apparent shift from no chilling injury (blotchy red colouration) after 1 or 3 d to injury after 5 or 7 d at 7.5 °C. The same could be true for describing increased ion leakage as an indicator of chilling damage. Saltveit (1989) found that chilling does not immediately increase the rate of ion leakage from tomato pericarp disc; rather it causes a progressive increase in permeability over a few days of chilling.

If this time-temperature threshold model for each symptom is true, it is possible that each symptom may have an independent response to IW. In the present study, IW at 6 °C enabled tomatoes to develop full red colour. However, the same cycles of IW at 2.5 °C did not enable fruit to develop full red colour but did reduce decay (Chapter 4). These results agree with Artés and Escriche (1994) who found that storing tomatoes at 9 °C with cycles of IW reduced CI but the same cycles in fruit stored at 6 °C did not. Nonetheless, their results indicated that IW at 6 °C did reduce pitting during storage, if not other symptoms. These results reinforce the idea that different CI symptoms have different threshold temperature and respond differentially to IW.

Since IW can recover the metabolic dysfunction and reverse visible symptoms at 9 °C but not at 6 °C (Artés and Escriche, 1994) or in our study at 6 °C but not at 2.5 °C, transition from reversible to irreversible damage seems to take different periods of time for different symptoms. Therefore, if different symptoms have different transition periods, it is not always possible for a particular IW time-temperature matrix to alleviate all symptoms specifically for those symptoms that have developed before IW is applied. For instance, since cycles of IW to 20 °C for 1 d every 7 d at 2.5 °C did not usually allow fruit to turn full red after storage (Figure 4.2), perhaps cycles of IW at 2.5 °C needs to be employed more frequently than what was applied in the study i.e. possibly after every 3 d. However,

as IW is itself a cumbersome technique for commercial application, more frequent warming is simply an impractical suggestion.

Chilling sensitive fruit develop a series of symptoms and each symptom has an independent time-temperature threshold to appear. Consumer perception of 'critical threshold level' may, however, change in a commercial situation depending on socio-economic or cultural consumer attitudes. For instance, loss of flavour is an important quality attribute for consumers from high-income affluent households but not necessarily for consumers coming from poor socio-economic conditions. For them, having a red colour tomato may be more important than tomatoes of good red colour with high flavour. The commercial consequence of varying thresholds for time-temperature dependent initiation of CI symptoms is possibly different in different markets. Here tomato is just an example; it could also apply for other chilling sensitive crops. In addition to consumer segregation, the concept of threshold time-temperature for a CI symptom may vary depending on the industry such as between fresh tomato and processing industry. For instance, tomatoes with pitting or chilling-induced 'mild' texture alteration can be presumably processed without overall quality (inhibition of red colour development, induction of mealiness) being compromised and therefore those tomatoes can be stored for longer period before processing.

7.3. Ripening versus chilling

Mechanisms of many chilling-induced physiological alterations can be confounded with normal ripening-associated changes since some of the processes occurring during development of CI are similar to those which occur during ripening and senescence. For example, during ripening, fruit soften and show increased disease susceptibility, increased ion leakage, and increased ethylene production. Most of these, if not all, physiological and cellular changes may occur after chilling-induced damage. Therefore, it is important to differentiate the physiological or cellular alterations accompanying ripening from the changes that reflect chilling damage.

As an example, loss of flavour during low temperature storage cannot always be used as an indicator of chilling damage. Many studies use mature-green tomatoes and store them at low temperature for a certain period of time and describe flavour loss as chilling-induced damage. However, this flavour loss at low temperature does not necessarily indicate

chilling-induced damage; instead it could be simply delayed ripening or slow ripening as a result of exposure to low temperature. It could be argued that the excessive softening described in the literature as chilling-induced damage was the same phenomenon. Excessive softening is reported as a CI symptom in tomatoes over a wide range of temperatures (2.5 to 10 °C) (Figure 7.1). Since ripening can initiate softening, and not all studies carefully separate chilling-induced softening from ripening-related softening, it is difficult to determine if the reported chilling-induced softening (especially at warmer temperature such as 10 °C) is not confounded by ripening-related softening. Similarly, this may be case for determining chilling-induced increased ion leakage. Fruit maturity has a large influence on membrane permeability and solute leakage. A ripening related increase in electrolyte leakage is well documented in many crops (Lewis and Martin, 1969). Therefore, an increased rate of ion leakage due to chilling stress can be confounded with ripening related increase in ion leakage.

An attempt was made in the present study to differentiate these two physiological processes using colour as an indicator of ripening. An example is distinguishing chilling-induced softening from ripening-related softening. By plotting firmness data (as a dependent variable) against colour (as an independent variable), significant different trajectories in fruit stiffness were observed between fruit stored at 2.5 or 6 °C and 8 or 20 °C (Figure 7.2A). It is suggested that loss of stiffness of fruit stored at 8 or 20 °C was coordinated with ripening (colour change), whereas fruit at 2.5 or 6 °C failed to coordinate softening with colour change, thus softening was chilling-induced (2.3.3).

However, there is another way of interpreting these data (Figure 7.2B). Since fruit softening is also an indicator of ripening, by using softening (stiffness) as an independent variable against colour, the dependent variable, it can be argued that at the same maturity (determined by fruit stiffness), fruit stored at 2.5 or 6 °C and 8 or 20 °C showed different trajectories in colour change, so therefore failure to achieve normal red colouration is a result of the “chilling damage”, but the softening is not. Therefore, it is difficult to determine if this “excessive softening” occurring without ripening (indicated by colour change) is chilling damage or a “failure to change in colour” during ripening (indicated by softening) is actually chilling-induced damage. But either way the fact that the trajectory is “different” from 8 or 20 °C is what tells us that these fruit stored at 2.5 or 6 °C are indeed injured.

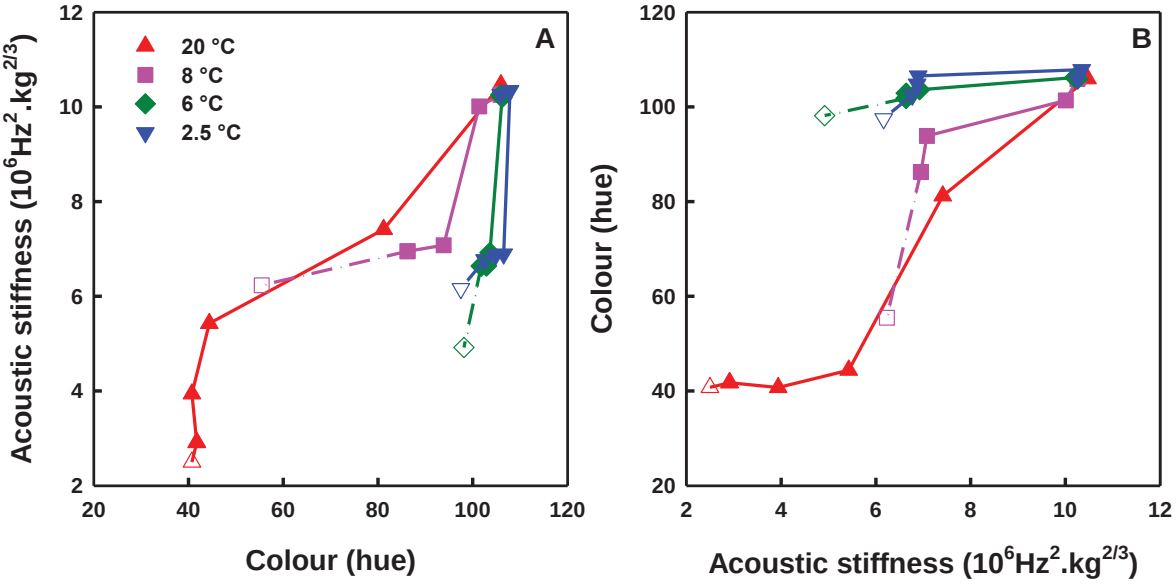

Figure 7.2 Differentiation of physiological changes during ripening (colour) and chilling damage (indicated by loss of acoustic stiffness) of mature-green tomatoes stored at 20 (▲), 8 (■), 6 (♦) or 2.5 °C (▼) (A). Dashed lines with open symbols indicate transfer of fruit from chilling conditions to 20 °C. Same colour data now used as a dependent variable and plotted against stiffness (an indicator of ripening) as an independent variable (B).

Using colour (an indicator of ripening) to an attempt to separate chilling induced physiological changes from ripening-associated changes, therefore, has its own limitations and the challenge remains as to how to clearly differentiate chilling injury symptoms from ripening-related development. It may be possible to use some unambiguous marker of chilling damage, e.g. *Alternaria* severity (Figure 7.3) or pitting, to separate chilling-induced physiological changes from events which are ripening-related. In that case, using both colour and softening as an independent indicator of ripening defines *Alternaria* severity as the chilling damage which is independent of ripening Figure 7.3.

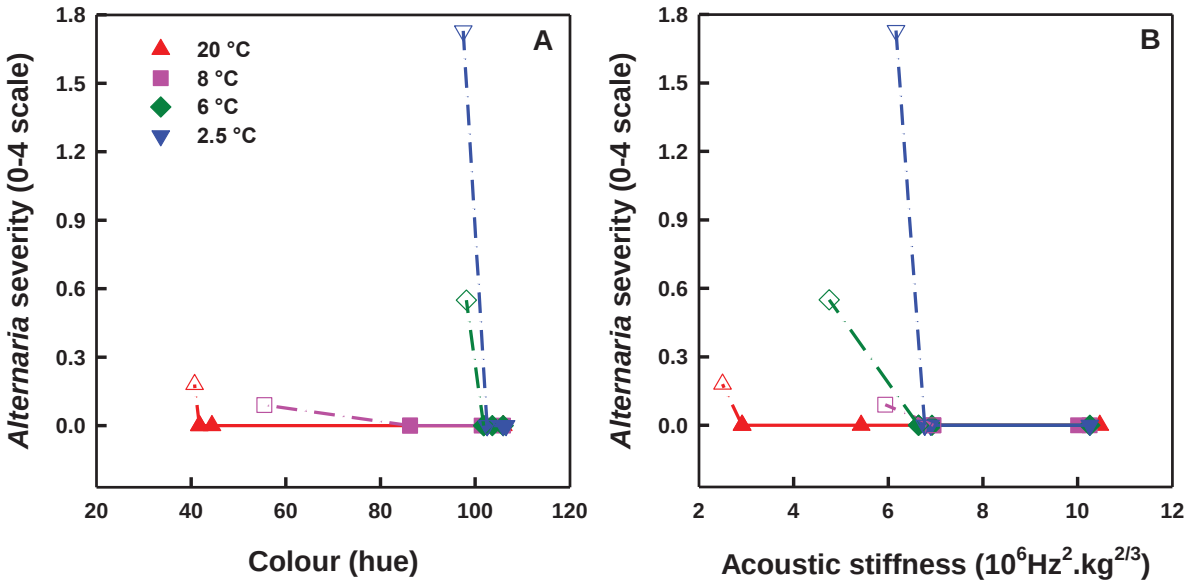

Figure 7.3 ***Alternaria*** **severity as an unambiguous chilling damage marker plotted against colour (A) or acoustic stiffness (B), as an indicator of ripening of mature-green tomatoes stored at 20 (▲), 8 (■), 6 (♦) or 2.5 °C (▼). Dashed lines with open symbols indicate transfer of fruit from chilling conditions to 20 °C.**

Genomics study may help us to distinguish physiological abnormality due to chilling from that related to ripening, but the choice of mutants would be critical. Studies using ripening mutants (e.g. *Cnr*, *Nr*, *rin*, *nor*) may not help because these mutants mostly do not attain normal red colouration during normal ripening. Perhaps studying chilling in antisense-fatty acid desaturase plants may provide a way to distinguish ripening from chilling in future. Nishida and Murata (1996) demonstrated that concentration of unsaturated fatty acids can be modified by the action of different enzymes like acyl-lipid desaturases and glycerol-3-phosphate acyltransferase (GPAT), thus subsequently manipulating the chilling sensitivity of plants. Murata et al. (1992) used a gene of chloroplast located GPAT (important for the rate of desaturation) from *Arabidopsis thaliana* and used *Agrobacterium* mediated transformation to express it in tobacco.

Proteomics studies may allow separation of physiological changes of chilling from those which are ripening-associated. Proteins are major functional determinants of cell machinery and proteomics approaches provide a key tool to identify functional proteins responsive to low temperature stress (Page et al., 2010) or proteins that may act to maintain cellular homeostasis under cold-stress (Vega-García et al., 2010). It may be possible to find

a marker, where for example, some proteins are induced only during the time of environmental stress (e.g. HSPs during heat treatments) but not during ripening. More importantly, if such kinds of proteins show varied expressions during IW treatment, they could be a useful marker of the avoidance of stress. Recently, Sanchez-Bel et al. (2012) indicated that aldose-1-epimerase, might be a possible candidate for a protein marker, as it seemed to have an important role in low temperature tolerance. It may be possible to get a signal indicating the onset of almost irreversible damage and activate IW at that time. Overall, these techniques provide scientists with an exciting opportunity to elucidate the time course and cause of chilling injury and resolve this problem in future.

7.4. IW reduces CI – does it influence the 'primary cause' or only delay expression of symptoms?

Chilling injury can be considered as consisting of two processes – primary and secondary events. The primary event is the initial, rapid response to the chilling temperature; this stage is reversible. Sustained primary damage causes a cascade of secondary effects after a period of time. It seems obvious that IW does not influence the initial events (which may begin within minutes of chilling) but it may delay the onset of secondary events (i.e. delaying the onset of visible symptoms) and prevent secondary events from becoming irreversible (thereby allowing the tissue to ripen normally after cold storage). There have been a number of attempts to explain the biochemical and physiological mechanisms underpinning these primary and secondary events.

Changes in lipid composition of membranes (decrease in phospholipids, decrease in unsaturated fatty acids) cause a decrease of fluidity that makes the membrane dysfunctional and decreases functionality of proteins associated with it (Lurie et al., 1997; Staehelin and Newcomb, 2000). Therefore, membranes that contain a higher degree of unsaturated fatty acids (linoleic and linolenic acid) are more chilling resistant (Lee et al., 2005; Zhang and Tian, 2009) than chilling sensitive plants that usually contain much a higher proportions of saturated fatty acids (Murata and Nishida, 1990). Increased concentrations of unsaturated fatty acids were reported in heat-treated tomatoes compared to control (Whitaker, 1994), and it is possible that the heated fruit had more fluid membranes (Lurie et al., 1997). It is suggested that IW mimics heat treatment in influencing lipid composition in the cellular membranes. Since intermittent warming could maintain high level of phospholipids and increase degree of unsaturation of fatty acids

concentrations during a rapid readjustment of metabolism (Wang, 1982), IW probably helps the membrane to return the lipids to a less gel-forming condition and undoes the primary response.

One of the earliest theories related to the cause of CI proposed that accumulation of toxic metabolites was caused by a temperature induced imbalance in metabolism (Nelson, 1926 as cited in Saltveit and Morris, 1990) and the inability of chilling sensitive plants to detoxify these compounds at low temperatures (Caldwell, 1990). Subsequent warming of fruit apparently induced higher metabolic activity that removed excess intermediates and replenished deficiencies that developed during chilling (Wang, 1993). For instance, reduction of superficial scald in cool-stored apple by intermittent warming could be associated with either inhibition of toxic compounds such as α-farnesene and its oxidation products or enhanced catabolism of conjugated trienes during warming periods (Alwan and Watkins, 1999; Rudell et al., 2011). Since IW of fruit induced higher metabolic activity in each warming occasion (Figure 6.6 and Figure 6.11), it has the potential to reverse toxic metabolites and undo the primary response.

While IW may have direct effect on removing toxic metabolites build up during chilling by inducing higher metabolic activity, it may have an indirect effect of quenching other toxic compounds such as ROS by enhancing antioxidant activities and subsequently reducing CI. Several enzymes are involved in scavenging free radicals in plant defence systems. Different postharvest treatments (e.g. heat treatments) are able to manipulate antioxidant systems in tissues of fruit and vegetables (Soto-Zamora et al., 2005). Wang and Baker (1979) reported that IW increased the contents of polyamines (spermidine and spermine) and stimulated activities of free radical scavenging enzymes in cucumbers and sweet peppers. In the present study, IW enhanced red colour development (Figure 4.2); Tonucci et al. (1995) found that red colouration in tomato was highly correlated with lycopene content, a potent quencher of ROS (Di Mascio et al., 1989). Tomatoes develop red colour as chloroplasts change to chromoplasts. Two main electron transport systems in plant cells are located in chloroplasts and mitochondria and usually responsible for the generation of oxygen free radicals during chilling stresses (Purvis and Shewfelt, 1993). If the chloroplast is a source of ROS and the chromoplast is not, then it is possible that reduction of CI by IW in the present study was through stimulating an antioxidant protection mechanism that suppressed chilling-induced oxidative stress and quenched ROS.

Synthesis of HSPs during different heat treatments is well documented in the literature and many researchers attributed acquisition of low temperature tolerance to induction of these HSPs (Lafuente et al., 1991; Sabehat et al., 1996). However, no information could be found in the literature about synthesis of HSPs in response to intermittent warming. Alleviation of CI and induced chilling tolerance by heat treatments usually involve exposure of plant tissues to a relatively higher temperature than the temperature employed during intermittent warming. Therefore, it is not clear if IW is perceived as a heat treatment by the plant cells. Lafuente et al. (1991) indicated that exposure of plant tissues to a sudden jump of temperature about 5-10 °C above the normal growing temperature may well be enough to induce the synthesis of HSPs. Therefore, it is possible that warming the fruit intermittently during low temperature storage may stimulate synthesis of HSPs that could be involved in recovery from chilling stress and confer protection from subsequent chilling stress. This is a specific and testable hypothesis which has not been pursued in this thesis and needs to be investigated in future work.

Overall, it is possible that IW can repair the primary damage and protect the tissue against subsequent low temperature. IW could reverse phase changes of the membranes, allow repair of damaged membranes and organelles, restore metabolic imbalances and allow metabolism of toxic metabolites. Periodic warming of tissues could maintain high levels of phospholipids, increase degree of unsaturation of fatty acids, increase the concentration of spermidine and spermine, and stimulate activities of free radical scavenging enzymes. Heat treatment induces HSPs, suppresses oxidative activity, and maintains membrane stability. All of these processes may contribute to the beneficial role of IW in enhancing chilling tolerance of tissues.

7.5. Reduction of CI by IW has both ethylene dependent and independent components

IW alleviates CI in tomato although its effectiveness was dependent on storage temperatures and nature of CI symptoms (Chapter 4). IW stimulated ethylene production and advanced red colour development during and after storage. However, when IW-stimulated ethylene response was arrested by 1-MCP, fruit remained green during storage. Although those 1-MCP treated fruit changed colour during the post-chilling period at 20 °C possibly by synthesising new ethylene receptors, fruit failed to achieve full red colour

and remained blotchy yellow-orange in colour, indicating advancement of red colouration in IW fruit was ethylene dependent (Chapter 6).

However, blocking ethylene action by 1-MCP did not prevent all beneficial effects induced by IW. Reduction of CI by IW could be ethylene independent depending on the nature of symptoms. IW at 2.5 °C did not stimulate ethylene production during storage yet reduced decay significantly after storage (Table 4.1). More direct evidence comes from IW fruit treated with 1-MCP which also showed reduced decay (section 6.3.1.1 and 6.3.2.1). These results indicate that the mechanism for chilling injury protection by IW cannot be solely attributable to ethylene action.

Altered red colour development, which is the most visible CI symptom in tomatoes, was perhaps due to a deficiency in the synthesis of lycopene (Hamauza and Chachin, 1995). IW at 6 °C resulted in tomatoes with greater red colour development than constant low temperature during storage with the fruit subsequently developing full red colour suggesting that enzymes required for lycopene synthesis were functioning during and after cool storage. IW stimulated enzyme activities related to ethylene production in reducing woolliness in peaches (Zhou et al., 2001). Since enzyme reaction velocity is approximately doubled with each increase in temperature of 10 °C (Caldwell, 1990), it is likely that during each IW regime temperature rise to 20 °C, chemical reactions proceeded faster than constant low temperature and this caused enhanced activities of enzymes involved in lycopene synthesis. Perhaps the beneficial effect resulting from enhanced enzyme activities continued after fruit were returned to 6 °C. However, the very low temperature (2.5 °C) possibly prevented the benefit of IW being realised because enzyme activities were severely impaired. Application of exogenous ethylene to fruit stored at 2.5 °C did not induce full red colour, indicating fruit were physiologically unable to respond to ethylene at this temperature. Perhaps benefits of IW still require some metabolism to be active in storage and 2.5 °C is simply too cold for most metabolism.

In a response similar to that of IW, intermittent application of ethylene (IE) induced fruit at 6 °C to develop full red colour and reduced CI on post-chilling transference to 20 °C. Fruit stored under the same IE treatment at 2.5 °C, however, failed to increase in red colouration but had reduced decay. While different CI symptoms have different threshold temperatures, this result suggests that different symptoms responded differentially to IE.

Kader and Morris (1975) reported that exposure of mature-green tomatoes to ethylene or ethephon following storage at 0 or 5 °C reduced abnormal ripening but not the severity of other chilling symptoms during post-storage ripening at 20 °C. Importantly, since IW reduces decay without influencing ethylene action and on the other hand IE also reduced decay, it seems that mechanisms of chilling-induced decay reduction in tomato were both ethylene dependent and independent.

Results in this study suggest that tissue held at 6 °C perceived ethylene and transduced the signal during cool storage whereas below 6 °C, ethylene binding capacity decreased. Jiang et al. (2004) suggested that ethylene binding by banana stored at 3 or 8 °C decreased with reduced storage temperature and thereby resulted in failure to ripen. Although ethylene binding capacity was decreased with lower temperature, ethylene perception is by no means completely inhibited. While the amount of ethylene perceived by the tissue at 2.5 °C was not enough to attain red colouration, it was sufficient to activate defence systems against decay (Chapter 6). Additionally if ethylene receptors were already blocked by 1-MCP, fruit failed to "activate" the defence mechanism against decay and showed increased decay susceptibility (Chapter 5). This confirmed the beneficial role of ethylene in reducing CI in tomato, although effectiveness was dependent on ethylene responsiveness of fruit tissue. Importantly, inconsistent results reported in literature regarding the beneficial effect of ethylene against CI were probably a result of using different low temperatures during ethylene treatment resulting in differences in ethylene perception and/or signal transduction by the tissues.

Since ethylene binding by the tissue can be decreased with lowering the temperature, it is possible that below a certain chilling temperature (in this case ≤ 6 °C), full benefit of ethylene can be achieved by applying ethylene in conjunction with exposing fruit to a short period of higher temperature i.e. removing chilling stress for the period of ethylene application. For instance, the IW+IE treatment used here indicated that IE supplied to fruit at constant cool temperature (2.5 °C+IE) did not advance red colouration whereas IE supplied during a warming period (IW+IE) resulted in full red colouration. In a commercial situation, it may be possible to turn off the refrigeration system during ethylene application for a certain period (e.g. 24 h) and turn on the system after that period. Additionally, in theory it may help to save energy.

While IE at constant 6 °C helped fruit to develop red colour, phenotypic changes (red colouration) only appear once fruit are transferred to a non-chilling temperature. Fruit remained green both in control (6 °C) and 6 °C+IE treatments during cool storage, but on post-chilling removal to 20 °C, fruit in 6 °C+IE fruit developed normal red colouration but control fruit without ethylene developed blotchy red colouration. However, the role that ethylene played to induce that positive response (as seen by red colouration later on) in 6 °C+IE fruit is not obvious. Ethylene acts as a regulator of ethylene-dependent processes in ripening including red colour development. In this study, it is possible that ethylene had a 'priming effect' on building up precursors of lycopene in mature-green tissue during exposure to low temperature enabling fruit to develop red colouration once the chilling stress was removed. On the other hand, control fruit was not 'primed', and therefore developed blotchy red colouration once chilling stress was removed. This implies that once ethylene is perceived by the tissue, ethylene transduces the signal to the chloroplast to convert to chromoplast (i.e. initiate biochemical reaction for chlorophyll degradation and lycopene synthesis). However, since "chilling stress" was still present, tissue was not metabolically active enough to initiate enzyme activities associated with chlorophyll degradation or lycopene synthesis.

The results reported here indicate that IE has the potential for application in commercial situations and this technique could be an alternative to IW. More importantly, IE at 6 °C caused tomatoes to develop full red colour once they were removed from cool storage (without colour change and significant softening during storage). In commercial situations, it could be an ideal technique because handlers of fresh tomatoes predominantly want tomatoes to remain firm and green (non-ripened) during cool storage but develop full red colour without risk of CI once fruit are displayed on a supermarket-shelf. In that case, application IE would be more advantageous than IW since fruit subjected to IW starts to develop red colour during cool storage and softens relatively more quickly than IE-treated fruit.

While IE showed promise in reducing CI in tomato, it challenges us with further research questions to answer in future. Based on the work presented in this thesis, a number of future lines of enquiry are possible. Of course, some of these questions extend our knowledge in elucidating CI phenomenon, while others have clear commercial potential and hence provide exciting opportunities for future research.

7.6. Future studies

7.6.1. Role of exogenous ethylene in reducing CI

These results indicated that intermittent ethylene application can reduce CI in fruit stored at 6 °C. However, effectiveness of ethylene supply is a function of storage temperature as reported in case of IW. In addition, ethylene application dose, timing and/or cultivar could be important factors. Therefore, there is a need to optimise an effective intermittent ethylene (IE) treatment for commercial purposes. Moreover, the effect of IE on consumer perception of quality needs to be investigated.

Any living cell exposed to a stress condition senses the 'stress' and transduces a signal (Stanley, 1991). Tissue then responds to acclimatise to the modified condition by maintaining cellular homeostasis and changing strategy to cope with that stress by redesigning its cellular structure physically, modifying lipid compositions or altering structure or function of enzymes involved in key metabolic reactions (Caldwell, 1990; Lurie et al., 1995). However, if the chilling stress continues, after a certain period irreversible damage occurs and symptoms develop. Therefore, it should be possible to find some proteins involved in the cold stress response mechanisms, and those that indicate damage (i.e. that the cell is moving to irreversible phase). If a signal can be found just prior to the onset of irreversible chilling damage can be found, then it may be possible to activate processes at that stage (e.g. activate IW or IE supply) and return the damage to the reversible phase.

Malondialdehyde (MDA), a secondary end product of polyunsaturated fatty acid oxidation, could possibly be used as a marker; it is usually considered as an indicator for damage of the structural integrity of membranes during chilling stress (Hodges et al., 1999; Zhao et al., 2006). Ethane evolution (as an indicator of lipid peroxidation) can be used also as a non-destructive marker of chilling damage (Kuo and Parkin, 1989). Similarly, quantification of ROS (e.g. singlet oxygen, superoxide radicals, hydrogen peroxide) production possibly could be used as a marker of chilling stress. It is known that antioxidants protect cells against the damaging effects of ROS. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage and subsequently injury symptoms. It may be possible to find a stage when there is

an imbalance of the antioxidant concentrations and induction of ROS and that imbalance may give a signal. If it is possible to sense that signal by using some "electronic sensor", it would be possible to give an ethylene shot to return the fruit to its primary phase and hence reverse damage. This technology might be similar to the techniques used in 'dynamic controlled atmosphere' (Prange et al., 2003), in which periodic measurement of chlorophyll fluorescence is used to detect when the chlorophyll-containing fruit and vegetables are experiencing low O_2 stress. Whenever O_2 concentration drops below a threshold level, the fluorescence signal increases, O_2 is added and fluorescence returns to pre-stressed condition. This concept can be tried for applying the IE technique to prevent irreversible damage in future.

The recommendation of IE in reducing CI and extending storage life needs to be investigated in other sectors of tomato industry such as truss tomato and fresh-cut. In supermarkets truss-tomatoes are common and gaining increasing approval. Since calyx was removed for the tomatoes used in the present study, it is not known whether same beneficial effect of IE will be achieved in truss tomatoes. In tomatoes, the stem scar was found to be the main channel for diffusion of ethylene (Cameron and Yang, 1982); it is possible fruit without calyx will absorb ethylene differently than fruit with calyx. Therefore, truss tomatoes may not receive as much benefit as did loose tomatoes without calyx. Importantly, since ethylene accelerates chlorophyll degradation and tissue yellowing at warm temperatures, exogenous ethylene in cool store may accelerate truss (fruit stalk) and calyx yellowing or abscission of fruit from stalk resulting in an unattractive product for the consumers (Saltveit, 1999).

Some studies applied ethylene to determine if ethylene influenced CI or improved quality of fresh-cut red ripe tomato slices, but no conclusive results were found. Hong and Gross (2000) found that fresh-cut red-ripe tomato slices in packages with high ethylene concentrations stored at 5 °C had lower CI (indicated by water-soaked areas) than slices stored in packages with low ethylene concentrations. In contrast, Jeong et al. (2004) indicated that inhibition of ethylene action by 1-MCP reduced water-soaking incidence in tomato slices stored at 5 °C and they suggested that this was an ethylene-mediated symptom of senescence and not a CI symptom as proposed by Hong and Gross (2000). Additionally, Pangaribuan (2009) found that ethylene applied 2 d after slicing of 'turner' maturity tomatoes stimulated ethylene production and treated slices had an undesirable

effect of accelerated softening during storage at 5 °C. Since after excising, fresh-cut slices are already stressed, application of ethylene may result in water soaking, accelerate softening and cause other undesirable effects. It is possible that using mature-green tomato slices and applying ethylene in those slices would be beneficial. In the present study, intact mature-green fruit treated with ethylene did not develop red colour until after cool-storage. Fresh-cut green slices treated with ethylene may be ready-to-eat (red ripe) by the time they reach to the supermarket shelf because fruit are cut, slices will ripen faster than intact fruit.

7.6.2. Role of 1-MCP in influencing CI

1-MCP appears to reduce CI in many horticultural crops. Only a few studies have been undertaken using 1-MCP to influence chilling sensitivity during low temperature storage of tomatoes. In the present study, 1-MCP-treated mature-green tomatoes showed higher chilling injury and decay after storage at 2.5 °C than non-treated control. Therefore, our results and others (Jing and Zi-Sheng, 2011) indicated that application of 1-MCP before cool storage may not be appropriate for commercial use in tomato. However, since our study of 1-MCP was carried out only at 2.5 °C, it would be desirable to evaluate the effect of this treatment at different temperatures and with different cultivars.

7.6.3. IW and role of antioxidants to quench ROS

Low temperature stress can induce ROS. IW advances red colour development and increases accumulation of lycopene which is a potent antioxidant. ROS can lead to loss of cellular integrity, protein degradation, DNA damage and ultimately cell death. The role of IW-stimulated antioxidant in reducing ROS has not been characterised. It is possible that chill-stressed tomatoes may have a higher number of cells committed to cell death than fruit subjected to IW. Loss of integrity in the plasma membrane can be demonstrated either by using dyes such as propidium iodide, which are excluded by an intact membrane, or using fluorescent dyes such as fluorescein diacetate which are retained in the cells only if the membrane is intact.

7.6.4. Does IW induce HSPs during warming and induce chilling tolerance?

Exposure of plant tissues to high temperature stress at about 10-20 °C above the normal growing temperature stimulated synthesis of polypeptides known as heat shock proteins (HSPs) (Lafuente et al., 1991). Although the mechanism of cell protection against stress

activated by HSPs is unclear, there is much evidence showing that these HSPs may function as molecular chaperones (Saltveit, 2002). While low temperature can alter the solubility and folding properties of many proteins, HSPs assist to protect against stresses by controlling the proper folding and conformation of both structural and enzymatic proteins (Vierling, 1991). Therefore, HSPs bind to unfolded or denatured proteins and prevent cell damage (Saltveit, 2002), reduce chromatin condensation and DNA breakdown, and suppress oxidative activity (Wang et al., 2001).

No information could be found on the synthesis of HSPs in response to intermittent warming. Therefore, it is important to determine if there are any HSPs involved in conferring chilling tolerance caused by IW.

7.6.5. Can IW be used as a postharvest technique to retain the flavour of tomatoes during low temperature storage?

Loss of flavour is a subtle chilling injury symptom but nonetheless it does contribute to loss of quality (Kader et al., 1978; Maul et al., 2000). Since chilling alters lipid metabolism (Whitaker, 1994) and fatty acids are considered key precursors in the formation of tomato aroma volatiles (Wang et al., 1996), there is likely to be a direct connection between chilling induced lipid composition changes and aroma development. In addition, since volatile composition of tomato is related to fruit colour, especially those compounds which were derived by oxidation of carotenoids (Baldwin et al., 2000), and IW advanced red colour development, it is possible that an IW treatment can maintain tomato flavour during low temperature storage. As ethylene has a role in the biosynthesis of aroma volatiles and in regulating the essential enzymes in many crops, it is possible that IW stimulated ethylene can modify aroma compounds. It would be interesting to study the level at which IW stimulated ethylene production affects activity of these essential enzymes. It would also be important to determine whether the beneficial effect of IW treatment in retaining flavour (if it is found) is independent of ripening.

7.7. Conclusion

Exposing chill-sensitive crops to low temperature for too long induces a group of symptoms collectively defined as chilling injury symptoms. The present study successfully moved the research away from using collective term of “chilling injury” by differentiating

specific CI symptoms of tomatoes as influenced by different threshold temperatures. A storage temperature considered safe for mature-green tomatoes is reported as 12-13 °C. Below this threshold temperature, the present study indicated that different low temperature ranges affected tomatoes differentially as characterised by different CI symptoms. For instance, at a given storage duration, storage at 8 °C delayed but did not perturb red colour development, fruit maintained at 6 °C showed uneven blotchy red colouration and those at 2.5 °C showed a complete failure to develop red colouration and severe decay. This indicated that below a threshold temperature, chilling sensitive fruit develop a series of symptoms and each symptom may require an independent time-temperature threshold to appear.

IW has been shown to reduce CI and improve keeping quality of several horticultural crops (Saltveit and Morris, 1990). Results in this study suggested that while IW was effective in reducing CI, general recommendations for IW were not appropriate as the responses were highly dependent on production conditions and/or cultivar and because different symptoms of CI have independent responses to IW. The present study was undertaken to elucidate physiological responses by which IW reduces CI in tomato.

On each warming occasion IW stimulated ethylene production and advanced red colour development during and after storage. However, when IW-stimulated ethylene response was arrested by 1-MCP, fruit remained green during storage and blotchy yellow-orange after storage. It was concluded that IW-stimulated ethylene was required to reduce CI in tomato as also reported in some other climacteric fruit. However even after blocking ethylene response of IW fruit by 1-MCP, IW still reduced chilling-induced decay indicating that reduction of CI symptoms by IW was not solely attributable to ethylene action. IW possibly has some metabolic benefit independent of ethylene.

In the introduction of this study, it was proposed that a deeper understanding of the physical responses by which IW exerts its beneficial effects may suggest a novel technique which is more practical and may be quite different than IW. It was found that intermittent exogenous ethylene application was effective in reducing tomato CI, although this benefit was seen only with ‘mild’ chilling temperatures (6 °C). In support of this observation, inhibition of ethylene action with 1-MCP enhanced some aspects of CI, consistent with increased ethylene reducing CI (although that experiment was carried out only at 2.5 °C).

Although both IW and IE can reduce CI in tomato, applying IW is technologically challenging. In contrast, IE could be achieved relatively easily. Therefore, intermittent ethylene supply during cool storage could be used as an alternative approach to reduce tomato CI. The positive results of ethylene application may allow the tomato industry to store tomatoes for longer periods at chill-inducing temperatures and hence enable sea freight of tomatoes to new markets. It is anticipated that using IE treatment the 'storage time' would be expected to be at least 4 weeks at cool storage plus 7-10 d shelf life at the supermarket. This period of 'storage time' may allow sending tomatoes by sea to 'higher-priced' Asian markets such as Japan and Korea. Moreover, it may help to reduce any market glut and provide the tomato supplier much needed trade flexibility.

However, while ethylene showed promise as a tool to reduce CI and allow fruit to develop normal red colouration after cool storage, further research is required to determine optimum concentration, time and frequency of application, and efficiency when applied at a range of temperatures in order to derive a successful treatment that may have significant commercial application.

Additionally, while the findings are positive for a possible industry application, the magnitude of the positive effect in reducing CI needs to be determined for other tomato cultivars and presentations (e.g. with calyx on a truss or fresh-cut) and also for fruit growing in other locations. More importantly, if the recommendation of IE application is commercially adopted, in future, it will be important to investigate the effect of IE on sensory perception of tomato quality.

8. References

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Appendix I

Volatile analysis of Florida-grown mature-green 'Soraya' tomatoes stored under chilling (2.5 or 6 °C) and non-chilling (12.5 or 20 °C) temperature

Loss of aroma during low temperature storage is an important quality loss and occurs even before other visual injury symptoms may appear (Kader et al., 1976; Maul et al., 2000; Baldwin et al., 2011). Sugars and organic acids are important to sweetness and sourness and overall flavour intensity in tomatoes (Stevens et al., 1977). In the experiment reported in Chapter 2, fruit were analysed for aroma properties.

Total soluble solids of 'Soraya' tomatoes stored at different temperatures for 13, 27 or 13+7 d 20 °C (7 d post-storage period at 20 °C after 13 d cool storage) were no different (Figure A1 a). No significant effect of low temperature storage on total soluble solid was reported previously in tomato (Chomachalow et al., 2002; Javanmardi and Kubota, 2006; Luengwilai and Beckles, 2010). As such, 'normal' development of sugars in fruit is inhibited during postharvest cool storage (Maul et al., 2000; Díaz de León-Sánchez et al., 2009). However, unchanged TSS content due to chilling must be viewed with caution because individual sugars were not measured.

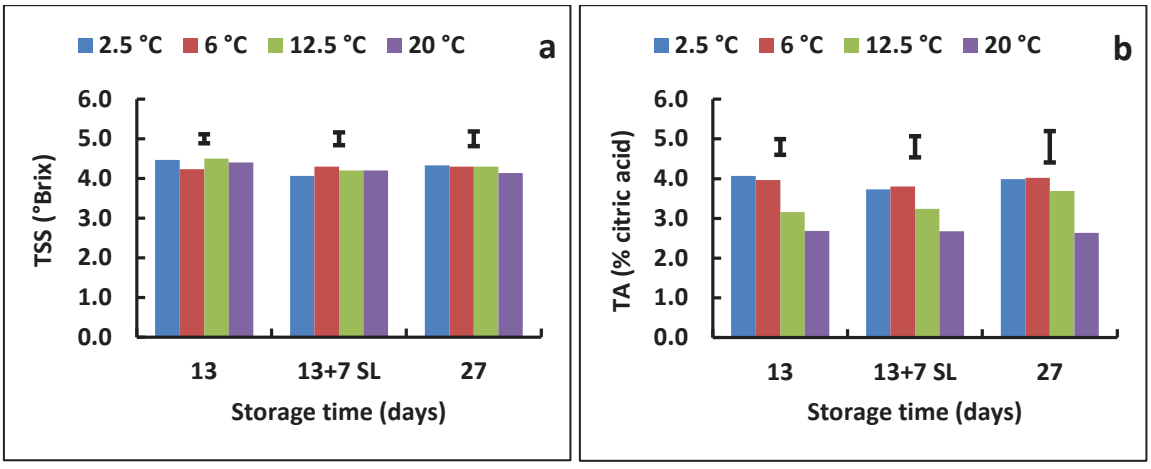

Figure A1 Total soluble solids (a) and titratable acidity (b) of mature-green tomatoes stored under different temperature treatments over time. Vertical bars represent LSD at α = 0.05 (n = 18).

For titrable acidity (TA), tomatoes stored at 2.5 or 6 °C had significantly higher acidity than fruit at 12.5 or 20 °C (Figure A1 b). Since decrease in some organic acids is typical during normal ripening in most standard tomato cultivars (Gómez et al., 2009), it was possible that fruit stored at 12.5 or 20 °C for 27 d were ripe (fruit also developed full red colour), whereas fruit stored at 2.5 or 6 °C failed to ripen (fruit did not develop full red colour). Exposure of tomatoes to chilling temperature prevented ripening-associated decline in acidity and adversely affected tomato flavour (Kader et al., 1986; Thorne and Efiuvwevwere, 1988). Maul et al. (2000) reported that tomatoes ('Somilar' and 'BHN-189') stored at 5 °C for 4-8 d had significantly higher titratable acidity and those fruit were rated significantly lower in tomato flavour and higher in sourness than fruit stored at 12.5 or 20 °C. However, it was not clear whether higher acidity due to chilling temperature reported by these researchers was a direct effect of chilling or indirect effect of failed ripening.

Principal Component Analysis (PCA), an unsupervised technique, showed a clear distinction between aroma compounds in fruit stored in chilled and non-chilled conditions during 27 d storage (Figure A2). Flavour profile in fruit stored at 12.5 or 20 °C was distinctly separated from fruit at 2.5 or 6 °C and without any differences between 2.5 and 6 °C-stored fruit. When loading plot was analysed, it was clear that the observed separation between chilled and non-chilled fruit was due to presence of some aroma compounds which predominantly characterise ripe-tomato flavour (red circle) and other aroma compounds which are clustered separately usually represent green tomato (green circle) (Figure A2).

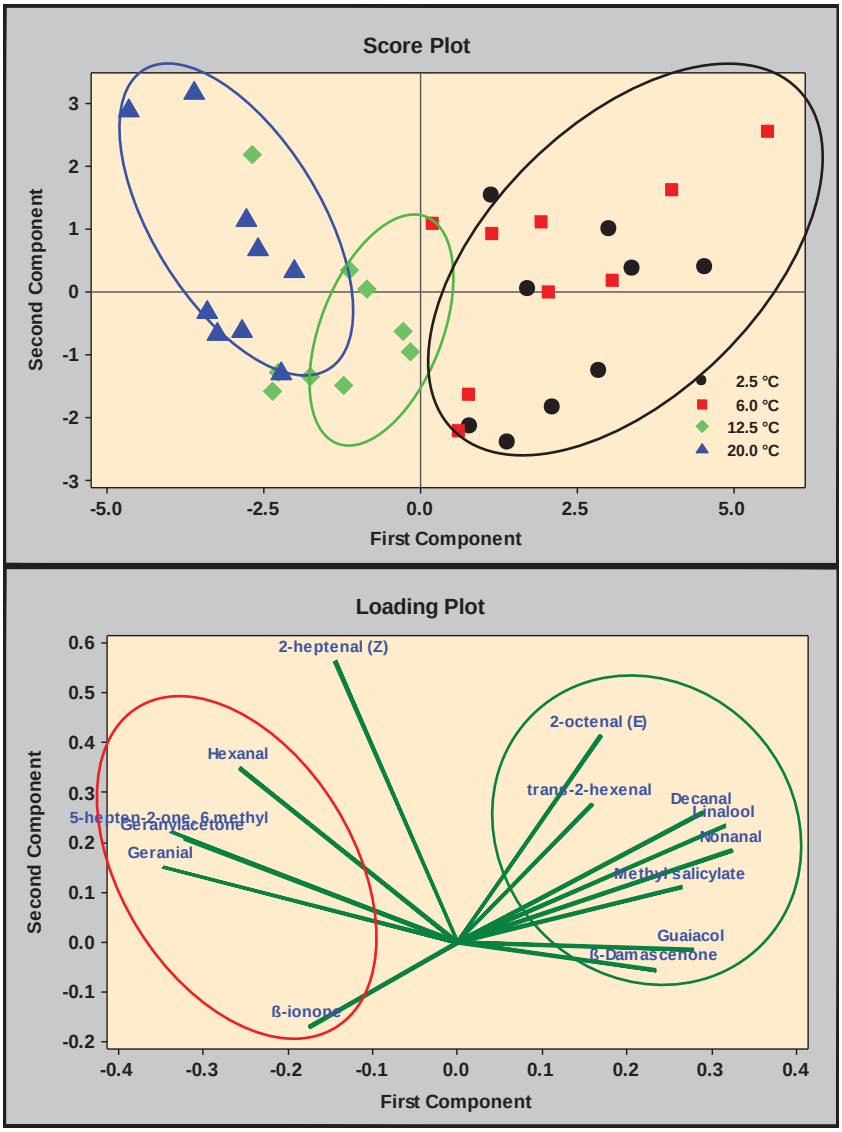

Figure A2 Principal Component Analysis (PCA) of flavour profile of mature-green tomatoes stored under different temperature conditions for 27 d.

This modification of aroma compounds in cool-stored tomatoes could be related to alterations in the availability of the precursors or enzyme activity in the biosynthesis of aroma compounds (Boukobza and Taylor, 2002). Since chilling alters lipid metabolism (Whitaker, 1994) and fatty acids are considered key precursors in the formation of tomato aroma volatiles (Wang et al., 1996), there is likely to be a direct connection between chilling induced lipid composition changes and aroma development. For example, concentrations of hexanal (which is produced as a result of cleavage of 13-hydroperoxide from linoleic acid), increased in tomatoes kept at 12.5 or 20 °C whereas it was not detectable in tomatoes kept at 2.5 or 6 °C during 13 or 27 d of storage (Table A1).

However, when fruit stored at 2.5 or 6 °C for 13 d were transferred to 20 °C, hexanal was still not detectable in fruit stored at 2.5 °C whereas 6 °C-stored fruit showed a significant increase in hexanal concentration to the same level as non-chilled fruit at 12.5 or 20 °C (Table A2). Reduced concentration of hexanal was found in tomatoes stored below 10 °C (Boukobza and Taylor, 2002; Díaz de León-Sánchez et al., 2009. As level of hexanal which mainly contributes sweetness intensity increases as the fruit ripen (Baldwin et al., 1991), lower level of hexanal in fruit stored at 2.5 or 6 °C suggested failure of ripening or delayed ripening and could not distinguish them further.

Volatile composition of tomato is related to fruit colour, especially those compounds which were derived by oxidation of carotenoids (Baldwin et al., 2000; Simkin et al., 2004). In this study, fruit stored at 2.5 or 6 °C had significantly lower level of carotenoid-derived volatiles such as 6-methyl-5-hepten-2-one, geranylacetone and β-ionone as compared to non-chilled tomatoes (12.5 or 20 °C) (Table A1). When all fruit were transferred to 20 °C following 13 d cool storage (13+7 d 20 °C), fruit at 2.5 or 6 °C still showed lower concentrations of these carotenoid-derived aroma compounds than fruit stored at 12.5 or 20 °C (Table A2). Reduced concentrations of carotenoid-derived volatiles were reported in tomatoes stored at 2 – 3 °C for 14 d (McDoland et al., 1999; Boukobza and Taylor, 2002). Since Baldwin et al. (2000) suggested that 6-methyl-5-hepten-2-one and geranylacetone were related to ripe aroma and “tomato-like” flavour, our results suggested that fruit held at 2.5 or 6 °C had disturbed metabolism and therefore failed to ripen or delayed ripening.

Concentrations of lignin-derived methyl salicylate, an important volatile responsible for the aroma wintergreen, was higher in tomatoes chilled at 2.5 or 6 °C than those stored at 12.5 or 20 °C (Table A1). Since methyl salicylate decreases with fruit ripening (Gao et al., 2007), significant lower concentration of methyl salicylate in fruit stored at 20 °C indicated accelerated ripening. The lack of significant change in methyl salicylate concentration in fruit stored at 2.5 or 6 °C during cool storage indicated that fruit stored at these low temperatures had disturbed ripening or delayed ripening (Table A2).

Table A1 Volatile compound concentrations[y] for mature-green 'Soraya' tomatoes stored for 13 and 27 d at different temperatures (2.5, 6, 12.5 or 20 °C).

			Day 13				Day 27			
Volatile	**Derived from**	**Odour[z]**	**2.5 °C**	**6 °C**	**12.5 °C**	**20 °C**	**2.5 °C**	**6 °C**	**12.5 °C**	**20 °C**
Characteristic ripe tomato volatiles										
Hexanal	Lipid	Green/grassy, green apple	nd	nd	12.71 a	9.61 a	nd	nd	13.37 ab	26.73 a
trans-2-Hexenal	Lipid	Green, almond oil	12.47 a	11.9 a	14.98 a	8.74 a	7.07 a	8.01 a	16.64 a	10.13 a
trans-2-Heptenal	Lipid	Green	0.73 a	0.81 a	1.24 a	0.95 a	nd	0.26 bc	0.76 ab	1.07 a
Geranial	Terpenoid	Fruity floral	nd	nd	2.99 a	4.57 a	nd	nd	1.70 b	3.30 a
ß-Damascenone	Carotenoid	Fruity/floral	1.16 ab	0.61 b	1.63 a	1.19 ab	1.18 a	0.98 a	1.40 a	0.65 a
Geranylacetone	Carotenoid	Sweet fruity	nd	nd	5.96 a	9.51 a	nd	nd	4.46 b	6.33 a
ß-ionone	Carotenoid	Fruity, fresh ripe	nd	nd	0.27 a	0.43 a	nd	nd	0.37 a	nd
6-Methyl-5-hepten-2-one	Carotenoid	Fruity, floral	0.47 b	1.12 b	7.35 a	7.46 a	0.42 c	0.31 c	4.46 b	8.28 a
Characteristic green tomato volatiles										
trans-2-Octenal	Lipid	Green leaf	0.60 a	0.76 a	0.49 a	0.84 a	nd	0.86 a	0.23 a	0.40 a
Guaiacol	Lignin	Medicinal	nd	0.26 a	0.38 a	0.17 a	0.70 a	0.64 a	0.23 a	nd
Linalool	Terpenoid	Fresh tomato, floral	5.29 ab	4.59 ab	3.85 ab	2.68 b	2.78 a	3.83 a	3.49 a	2.08 a
Methyl salicylate	Lignin	Wintergreen	4.68 ab	4.85 ab	3.08 ab	1.94 b	2.98 a	4.36 a	2.19 a	nd

Nonanal	Lipid	Fat, citrus green	1.62 a	2.22 a	1.37 a	1.53 a	1.29 a	1.96 a	0.93 a	1.07 a
Decanal	Lipid	Soap, orange peel	1.08 a	1.66 a	1.13 a	1.41 a	1.08 a	1.26 a	0.85 a	1.04 a

[y]Aroma volatile compound concentration means ($\mu L.L^{-1}$) with different letters across rows are significantly different at the 5% level according to Duncan's Multiple Range Test.

nd = non-detected

[z]Adapted from Baldwin et al. (2000)

Table A2 Volatile compound concentrations[y] for mature-green 'Soraya' tomatoes stored for 13+7 d 20 °C and 27+7 d 20 °C at different temperatures (2.5, 6, 12.5 or 20 °C). Fruit stored at 2.5 or 6 °C were infected with rot after 27 d, so no volatiles were analysed during 27+7 d 20 °C period.

			Day 13+7 d 20 °C				Day 27+7 d 20 °C			
Volatile	**Derived from**	**Odour[z]**	**2.5 °C**	**6 °C**	**12.5 °C**	**20 °C**	**2.5 °C**	**6 °C**	**12.5 °C**	**20 °C**
Characteristic ripe tomato volatiles										
Hexanal	Lipid	Green/grassy, green apple	nd	11.36 a	16.75 a	21.04 a			19.22 a	27.02 a
trans-2-Hexenal	Lipid	Green, almond oil	14.26 a	15.03 a	12.61 a	8.68 a			10.88 a	8.91 a
trans-2-Heptenal	Lipid	Green	0.54 a	0.58 a	1.05 a	1.06 a			0.91 a	1.00 a
Geranial	Terpenoid	Fruity floral	nd	0.40 c	2.87 b	4.10 a			1.98 a	2.51 a
ß-Damascenone	Carotenoid	Fruity/floral	1.31 a	1.45 a	1.37 a	1.62 a			0.69 a	0.24 a
Geranylacetone	Carotenoid	Sweet fruity	0.22 c	1.02 c	6.13 b	9.78 a			4.73 a	5.00 a
ß-ionone	Carotenoid	Fruity, fresh ripe	nd	nd	0.37 a	0.26 a			0.20 a	nd
6-Methyl-5-hepten-2-one	Carotenoid	Fruity, floral	0.81 c	1.96 c	6.65 ab	7.82 a			4.75 a	7.79 a
Characteristic green tomato volatiles										
trans-2-Octenal	Lipid	Green leaf	1.11 a	0.58 a	0.73 a	0.87 a			0.61 a	0.21 a
Guaiacol	Lignin	Medicinal	nd	0.24 a	0.17 a	nd			nd	nd
Linalool	Terpenoid	Fresh tomato, floral	3.78 a	3.58 a	2.68 a	2.39 a			2.02 a	1.25 a
Methyl salicylate	Lignin	Wintergreen	3.20 a	2.50 a	2.46 a	0.27 b			0.95 a	nd
Nonanal	Lipid	Fat, citrus green	1.82 a	1.84 a	1.51 a	1.49 a			0.78 a	0.81 a
Decanal	Lipid	Soap, orange peel	1.13 a	1.49 a	1.34 a	1.27 a			0.83 a	0.63 a

[y]Aroma volatile compound concentration means ($\mu L.L^{-1}$) with different letters across rows are significantly different at the 5% level according to Duncan's Multiple Range Test.

nd = non-detected

Overall, results reported here showed that tomatoes stored at 12.5 or 20 °C had higher concentrations of ripening related aroma compounds such as hexanal, 5-hepten-2-one-6-methyl, geranial, geranylacetone and β-ionone, whereas fruit held at 2.5 or 6 °C had higher concentrations of green tomato aroma such as methyl salicylate, linalool, guiacol, nonanal, and decanal. The effects of low temperature storage on tomato aroma volatiles were consistent as reported by other researchers (McDonald et al., 1996; Maul et al., 2000; Díaz de León-Sánchez., 2009). However, it is not clear whether lower concentrations of some aroma compounds found in cool-stored tomatoes were due to direct effect of low temperature stress or indirect effect of fruit's failure to ripen and subsequent reduction in flavour. In this study, it was impossible to determine whether altered volatile concentrations of chilled tomatoes compared to non-chilled control fruit were due to delayed or abnormal ripening.