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Effect of Ethylene and 1-Methylcyclopropene (1-MCP) on Color and Firmness of Red and Breaker Stage Tomato Stored at Different Temperatures

¹Tigist Nardos Tadesse, ²Brian Farneti and ²Ernst Woltering

¹Jimma University College of Agriculture and Veterinary Medicine, P.O. Box 1643, Jimma, Ethiopia

²Horticultural Supply Chains Group, Wageningen University, The Netherlands, Droevendaalsesteeg 1, 6708 PD Wageningen, Netherlands

Corresponding Author: Tigist Nardos Tadesse, Jimma University College of Agriculture and Veterinary Medicine, P.O. Box 1643, Jimma, Ethiopia

ABSTRACT

The red color of the tomato fruit during ripening is due to the presence lycopene, a compound known for its antioxidant property. This compound is influenced by temperature, ethylene and 1-Methylcyclopropene (1-MCP) treatment. It was believed that ethylene application at chilling temperature could help increase the amount of lycopene content of tomato fruits. In addition, application of 1-MCP was assumed to reduce the action of ethylene on production of lycopene content and decrease loss of firmness of tomato fruit under chilling temperature. Therefore, the objective of this experiment was to see the effect of ethylene and 1-MCP treatment on color and firmness of tomato fruit under different storage temperatures. A tomato variety (cv. Rotarno) grown under greenhouse condition harvested at red and breaker stage was stored at 4, 8 and 20°C to assess color and firmness. Tomato fruits were treated by ethylene (85.7 ppm) and 1-MCP (2.9 ppm) gases starting from the first day of storage throughout the experimentation period. The color and firmness of the fruits were measured by pigment analyzer and Zwick respectively. Results showed that there was significant difference in color value of ethylene treated breaker stage tomato. 1-MCP treatment reduced the color value of tomato fruits stored at 20°C more than the fruits stored at 4 and 8°C. However, 1-MCP treatment helped to reduce loss of firmness of the fruits. We conclude that external ethylene application on red tomato fruits stored at chilling temperature generally has no effect on the subsequent accumulation of lycopene or to maintain the loss of accumulated lycopene during chilling storage. However, external ethylene treatment on breaker stage tomatoes helps for red color development. In addition, 1-MCP treatment helps to reduce loss of firmness of the fruits.

Key words: Tomato, lycopene, antioxidant, ripening, storage, 1-MCP

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the most important and extensively consumed vegetable crops worldwide which is produced for its delicious fruit (Frusciante *et al.*, 2007). The fruit is categorized under climacteric fruit as the trigger of the many remarkable physical and chemical changes in the process of ripening is highly governed (influenced) by ethylene production (Lelievre *et al.*, 1997; Guilleu *et al.*, 2006; Lu *et al.*, 2010). As a climacteric fruit ripening of

tomato is highly dependent on ethylene (Alexander and Grierson, 2002; Hoerberichts *et al.*, 2002; Mir *et al.*, 2004; Hertog *et al.*, 2004). Ethylene is a cause for the autocatalytic character of the overall colour change of tomato fruit (Hertog *et al.*, 2004). Softening rate of tomato fruit depends on the rate of ethylene production (Jeong *et al.*, 2004; Lu *et al.*, 2010). Harvesting methods, postharvest handling and packaging influence the effect of ethylene on quality characters (Saltveit, 1999). Once the ripening of tomato fruit begins, internal ethylene concentration quickly increases to saturation level and application of external ethylene has no further effect on ripening. Therefore, the external ethylene concentration generally has no effect on the subsequent ripening of fruit that are progressed a few days into their climacteric (Saltveit, 1999). The same author reported that response of the tissue to ethylene application depends on the sensitivity of the tissue, ethylene concentration, duration of exposure and temperature (Saltveit, 1999).

Temperature is by far the most important environmental factor in the post-harvest life of tomato fruit because it has a tremendous influence on the rate of biological processes, including respiration (Mostofi and Toivonen, 2006; Akter and Khan, 2011). Development of red colour and softening of tomato fruit deepens on temperature. The optimal temperature for red colour development on tomato fruit is reported to be ranging between 16-26°C (Mostofi and Toivonen, 2006). The reports by Javanmardi and Kubota (2006), Toor and Savage (2006), Yang *et al.* (2009) also indicate that temperatures between 15 and 25°C favour high accumulation of lycopene than that of tomato stored at 5, 7 and 12°C. Toor and Savage 2006 report that lycopene content of tomato stored between 15 and 25°C is 1.8 fold higher than that of tomatoes stored under chilling condition. However, storage of tomato fruit at higher temperature is not advisable, as this will result in excessive weight loss and failure to ripen (Lurie and Klein, 1992).

According to Atta-Aly (1992), tomato fruit produce maximum amount of ethylene when the fruit is stored at 20°C, however increasing holding temperature above 20°C reduced ethylene production of the fruit. Elevated temperature inhibits ethylene synthesis and interferes with lycopene synthesis, because disruption of enzymes activity as a result of the higher temperature (Lurie *et al.*, 1996). pTOM13 has been identified as a cDNA clone encoding ACC oxidase (Hamilton *et al.*, 1990) that catalyzes the last step in the ethylene synthesis pathway.

1-methylcyclopropene (1-MCP) is a gaseous substance which averts ethylene binding to active sites causing delay in formation of colour (both lycopene accumulation and chlorophyll degradation), softening and ethylene production in tomato fruit (Hoerberichts *et al.*, 2002; Jeong *et al.*, 2004; Amodio *et al.*, 2005; Mostofi and Toivonen, 2006; Guillen *et al.*, 2007). 1-MCP being an ethylene receptor blocker is considered to be a potential commodity to be used commercially than other ethylene binding inhibitors in order to extend post-harvest life of tomato fruit even when storage temperatures are high (Amodio *et al.*, 2005; Mostofi and Toivonen, 2006). According to the report made by Mostofi and Toivonen (2006) and Hoerberichts *et al.* (2002). 1-MCP prevents the accumulation of the number of mRNA in response to expression of ACC synthase, ACC oxidase and ethylene receptor in tomato fruit. This in turn, has effect on ripening of the tomato fruit as the suppression of ACC-synthase and ACC-oxidase would result in temporary inhibition of red colour development. The study of (Opiyo and Ying, 2005), indicated that there is variation in response to amount of 1-MCP as well as cultivar for efficient control of ripening of tomato fruit. When compared to untreated fruits, a matured green tomato fruit treated with 1-MCP concentration of 0.07 and 0.11 $\mu\text{L L}^{-1}$ were found to produce less ethylene. However, those fruits which were treated with relatively less concentration (0.035 $\mu\text{L L}^{-1}$) of 1-MCP produced even higher ethylene than non-treated one and attained their climacteric peaks seven days after treatment while the climacteric peaks of fruits treated by relatively higher concentration (0.07 and

0.11 $\mu\text{L L}^{-1}$) delayed to at least 13 days after treatment. Therefore, the objective of this experiment was to investigate the effect of ethylene and 1-MCP on color and firmness of tomato fruits stored under different temperatures.

MATERIALS AND METHODS

Experimental materials: Freshly harvested, non-damaged, red and breaker stage round shaped truss tomato fruits (cv. Rotorno) with 60-120 g weight were used as a test material. This experimental cultivar grew in the Southeastern part of the Netherlands in greenhouse Rijkzwaan by using natural light. Twenty tomato fruits were used per treatment and each tomato was numbered to avoid mix-up in data recording during repeated quality parameters measurement in different storage time.

Experimental setup and treatments: The experiment was carried out in the laboratory of Horticultural Production Chain group at Wageningen University from May 2011 to June 2011. Three levels of temperatures (4, 8 and 20°C) were considered for the treatment and in all levels of temperatures fruits were treated by ethylene, 1-MCP and control (non-treated fruits) making the total number of treatments nine. Tomatoes treated by ethylene and 1-MCP were stored for 24 days in closed containers which did not have hole on their lid but have rubber stopper in the lid. The remaining three control treatments were stored in a different container that had a hole on the top of the lid.

Ethylene with the concentration of 85.7 ppm was injected into closed containers through a rubber stopper. 1-MCP with the concentration of 2.9 ppm was allowed to diffuse in to a closed container where fruits were stored. These gases were applied at the first day of storage and then supplied every day after measurement. During the storage time, color and firmness were measured at four, three and two, days interval in fruits stored at 4, 8 and 20°C, respectively in order to see variations among the treatments.

Parameters taken

Color: The color of the fruit was measured without destructing using CP pigment analyzer PA 1101. The method of Rutkowski *et al.* (2008) was employed after modification to collect a spectral from pigment analyzer in order to calculate two standard indexes: (1) normalized different vegetation index $[\text{NDVI } (I_{780} - I_{660} \text{ nm}) / (I_{780} + I_{660} \text{ nm})]$ to measure green coloration and (2) normalized anthocyanin index $[\text{NAI } (I_{780} - I_{550} \text{ nm}) / (I_{780} + I_{550} \text{ nm})]$ to measure redness. They indicated the color variation in tomato fruits stored at different temperature. In order to measure spectral value (in nanometer) from stored fruits three circular spots were made on the surface of fruits making the first on the blossom end where fruit number was written. The remaining two spots were assigned as spot 1 and spot 2 from which color was measured and averaged.

Firmness: Firmness was also measured without destructing the fruits using Zwick Universal Type Machine (Lana *et al.*, 2007). In this method, the tomato fruit was placed in a plastic ring with its stalk positioned perpendicular to the center of gravity. The firmness was determined by exerting maximum force to compress the tomato fruit down to 2 mm at 40 mm min^{-1} speed from lowering the probe until it touches the tomato skin. One spot was used from a fruit to measure the firmness repeatedly from each treated and non treated fruit until the end of experiment.

Data analysis: Analysis of variance for the repetitive measurement was used to determine variation among the treatments for the variables recorded. The data obtained from this experiment

was analyzed using GenStat 13th Edition (VSN International, 2010). The results from the analyzed data were used to observe variations existing among different treatments.

RESULTS

Effect of ethylene and 1-MCP treatment on color of red and breaker stage tomato fruits:

Color value of red tomato fruits treated by ethylene, 1-MCP and non-treated fruits (control) stored at 4 and 8°C decreased as the time passed throughout the storage period. However, ethylene, 1-MCP treated and control fruits stored at 20°C showed an increase in color value during storage period (Fig. 1). The application of ethylene on the fruits stored at 4 and 8°C did not show any significant difference in increasing color value when compared to non-treated (control) fruits

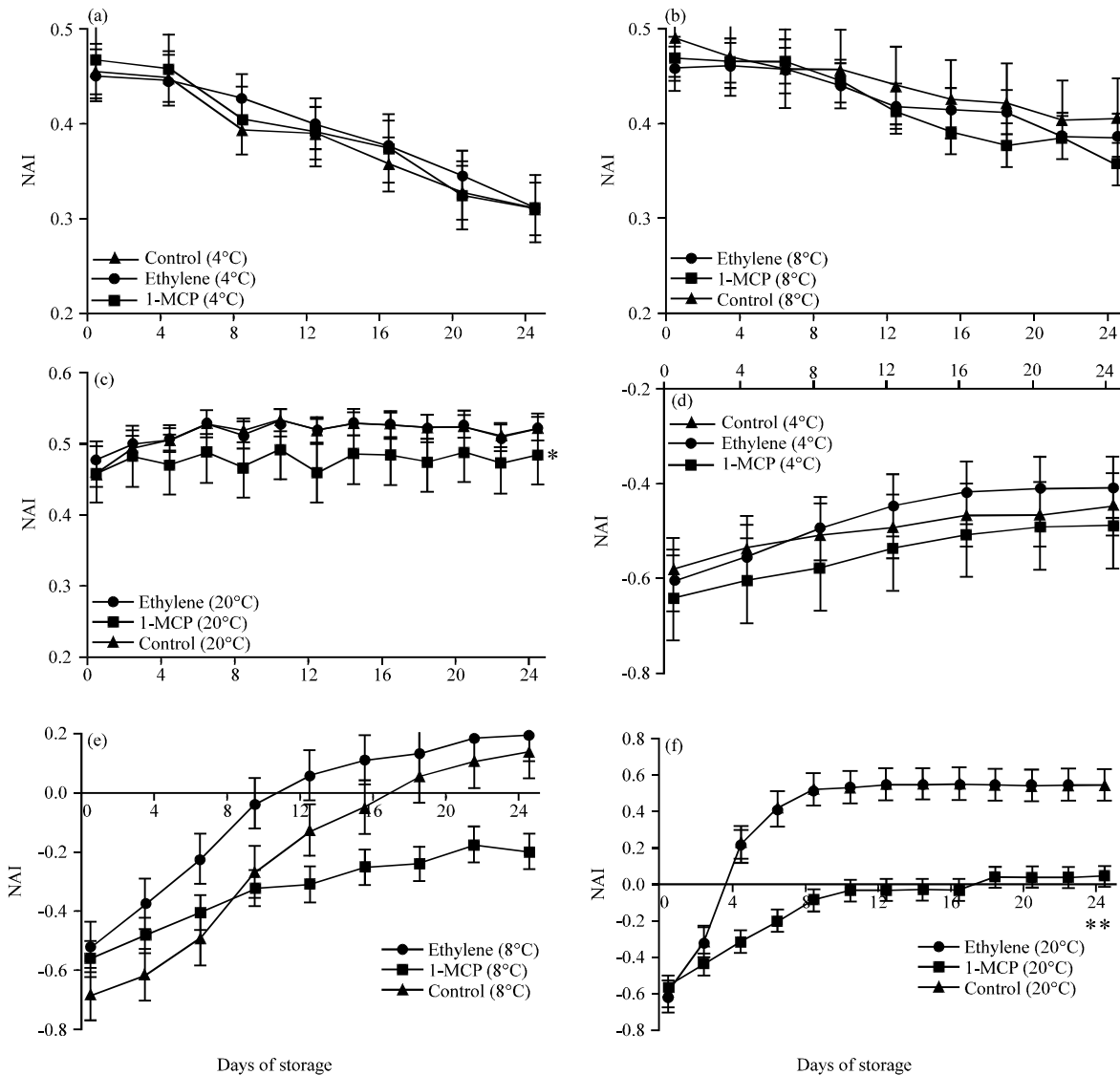


Fig. 1(a-f): Effect of ethylene, 1-MCP and control treatment effect on (a-c) Red and (d-f) Breaker stage tomato fruits stored at different temperatures. NAI: Normalized anthocyanin index, Data are Mean+SE, ***Treatments differ significantly and highly significantly at $p < 0.05$ and $p < 0.01$, respectively

(Fig. 1). In addition, application of 1-MCP on the tomato fruit did not result in decreasing of color value of the fruits compared to the control treatment (Fig. 1). On the other hand, the application of 1-MCP significantly ($p < 0.05$) decreased color value of red tomato fruits than ethylene treated and control fruits stored at 20°C (Fig. 1). In this study, ethylene did not show an increase in color value of the fruits stored at those three different temperature regimes.

The color value of breaker stage tomato treated by ethylene, 1-MCP and control treatments increased during storage at 4, 8 and 20°C (Fig. 1). Ethylene treated fruits scored the highest color value than 1-MCP treated and control fruits stored at 4 and 8°C (Fig. 1). However, ethylene treated and control fruits stored at 20°C scored the same color value throughout the storage time (Fig. 1). All 1-MCP treated breaker stage fruits stored at the three temperatures scored the lowest color value throughout the storage time while 1-MCP treated fruits stored at 20°C showed a highly significant difference ($p < 0.01$) compared with ethylene treated and control fruits (Fig. 1).

Effect of ethylene and 1-MCP treatment on firmness of red and breaker stage tomato fruits: The firmness of all ethylene and 1-MCP treated and control red tomato fruits stored at 4, 8 and 20°C for 24 days decreased during storage (Fig. 2). The fruits stored at 20°C scored the lowest firmness value at the end of experiment than the fruits stored at 4 and 8°C. Ethylene and 1-MCP treatment did not show significant differences in firmness of the fruits that were stored at 4, 8 and 20°C compared with control fruits (Fig. 2).

Similarly, breaker stage fruits showed a reduction in firmness throughout the storage period (Fig. 2). 1-MCP treated fruits scored the highest firmness value than ethylene treated and control fruits stored at those three temperatures. 1-MCP treated fruits were significantly higher ($p < 0.05$) in firmness than ethylene treated and control fruits stored at 20°C (Fig. 2). However, at 4 and 8°C, 1-MCP did not show a significant increment in firmness. On the other hand, ethylene treated fruits showed more loss of firmness than other treatments stored at 4 and 20°C (Fig. 2).

DISCUSSION

In this experiment, external ethylene application had no effect to maintain accumulated lycopene content of the fruits in red tomatoes stored at 4 and 8°C. Moreover, there were no differences in color value increment between ethylene treated and control red tomato fruits which stored at 20°C. This result is in agreement with the work of Saltveit (1999) who reported that once the ripening of tomato fruit begins, internal ethylene concentration will quickly increase to a saturation level and then application of external ethylene has no further effect on color value increment. Therefore, the external ethylene concentration generally has no effect on the subsequent ripening or accumulation of lycopene of fruit that are advanced a few days into their climacteric.

However, in our study external ethylene application on breaker tomatoes contributed for more color increment especially in fruits stored at chilling temperature (4 and 8°C) which is in agreement with Chomchalow *et al.* (2002). The authors reported that mature green as well as breaker stage tomatoes treated with ethylene attain the red stage earlier compared to untreated fruits stored at chilling temperature. In addition, the study of Chomchalow *et al.* (2002) indicated that ethylene application hastens fruit ripening and promotes uniformity of ripeness in tomatoes. Hence, external application of ethylene is more useful in green and breaker stage of tomatoes than red stage tomatoes to initiate lycopene development or for degradation of chlorophyll.

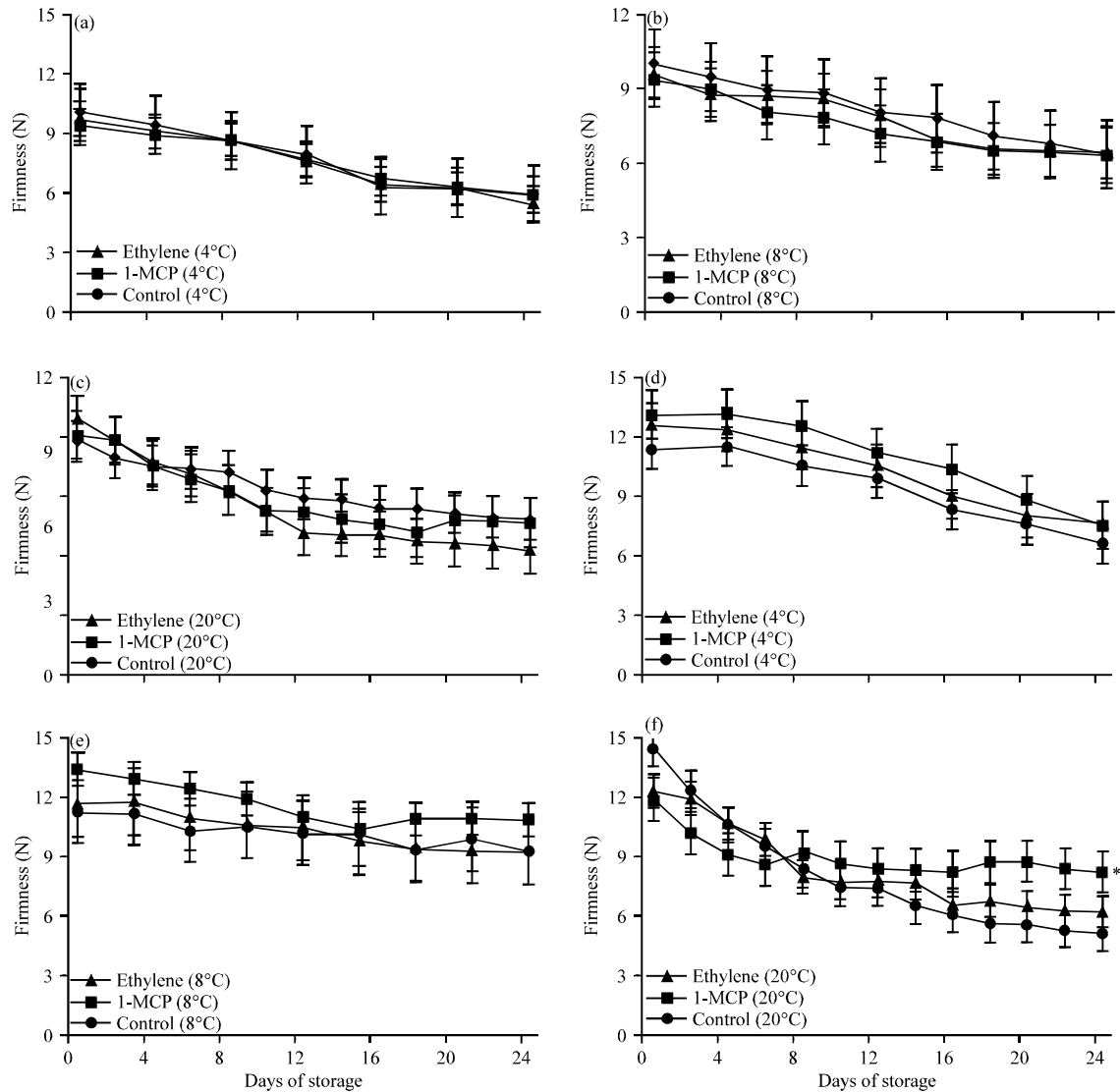


Fig. 2(a-f): Effect of ethylene, 1-MCP and control treatments on firmness of (a-c) Red and (d-f) Breaker stage tomato fruits stored at different temperatures, Data are means+SE, *Treatments differ significantly at $p < 0.05$

Accumulation of lycopene content was higher in fruits stored at 20°C than stored at 4 and 8°C. According to Lurie *et al.* (1996), tomato fruits develop red color normally when the fruits are stored at 20°C which is associated with peak ethylene production. Same researchers mentioned that the level of mRNA for ACC oxidase in tomato fruits increased when the fruits were stored at 20°C even after the tomatoes had passed the peak of their ethylene time. In addition during chilling, mRNA of ACC oxidase also increased in tomato. However, this increase was not evidenced by high ethylene production in these fruits. Therefore, according to Boller *et al.* (1979) the reduction of ethylene production at low temperature, despite the level of ACC oxidase mRNA, increased at chilling temperature. This is because of another step in ethylene synthesis was reduced by chilling, may be ACC synthase, the rate-limiting step in ethylene production. Besides, the Phytoene

Synthase (Psy) mRNA level paralleled quite closely to color development of the fruit in unchilled tomatoes (Lurie *et al.*, 1996). And also they reported that the fruits stored at chilling temperature increased messenger level slowly. This reduced the lycopene synthesis at low temperature.

1-MCP treatment on the red and breaker stage fruits stored at chilling temperature, especially stored at 4°C did not delay color value of the fruits as compared to the control fruits. According to (Mostofi and Toivonen, 2006), 1-MCP prevents accumulation of number of mRNA responsible for expression of ACC synthase, ACC oxidase and ethylene receptor in tomato. However, low temperatures suppress the activity of these mRNAs. This reduces the sharp increase in ethylene production in the fruits stored at chilling temperature. Due to reduction of ethylene production at this temperature, autocatalytic production of ethylene was also inhibited. According to Wills and Ku (2002) and Guillen *et al.* (2007), the highest percentage of blocking of ethylene production by 1-MCP only happens when there is higher ethylene production. However, 1-MCP treatment appreciably delayed change in the color value of red and breaker stage fruits that stored at non-chilling temperature. Atta-Aly (1992) stated that tomato fruit produces maximum amount of ethylene when it is stored at 20°C. Since the development of red color in tomato fruit is related to peak ethylene production, 1-MCP has more pronounced effect if there is high ethylene production.

In our result, ethylene treatment on red tomato fruits had no effect on loss of fruit firmness which was stored at chilling temperature when treated fruits were compared to non-treated ones. Chomchalow *et al.* (2002) support this finding by reporting ethylene application on red stage of tomato fruits had no any significant effect on firmness between fruits treated by ethylene before and after storing at 2.5 or 12.5°C. However, the same authors reported that in mature green and breaker stage tomatoes treated by ethylene before storage at chilling temperature, fruits became less firm in relation to fruits without ethylene treatment stored at chilling temperature. This implies that ethylene treatment is more important to mature green or breaker stage tomatoes than red tomatoes before storage at chilling to reduce loss of firmness because of chilling injury. Treating fruits by ethylene before storage of chilling temperature prevents chilling injury and prolongs shelf life of the fruits. As far as comparing loss of firmness in the treated and non-treated red and breaker stage fruits stored at higher and chilling temperature is concerned, the loss of firmness was higher in fruits stored at 20°C than 4°C and 8°C stored fruits. This result is supported by previous work of Artes and Escriche (1994) on tomato fruit. According to their findings, firmness of fruits decreased at higher rate during storage period at higher temperature than fruit stored at chilling temperature. This might be due to the reason that firmness can be maintained during storage at chilling temperature because same chilling temperature might inhibit softening process during post storage ripening. Even though storage at low temperature reduces softening process of tomato fruit, the reverse can happen at low temperature because of chilling injury (Lana *et al.*, 2005).

1-MCP treatment did not bring significant difference in loss of firmness of the red and breaker stage tomato fruits stored at 4 and 8°C. However, 1-MCP treatment reduced the loss of firmness significantly compared to control fruits which were stored at 20°C. Similar result was reported that 1-MCP showed a delay in softening fruits after 30 days of storage at ambient air temperature (20°C) in non-astringent persimmon (Ramin, 2008) and mango (Liu *et al.*, 2010). Normally, tomato is characterised by a rise in ethylene reaching climacteric peak. Ethylene triggers the ripening process with the acceleration of color change and softening. However, chilling temperature reduces ethylene production of fruits. Due to this reason, 1-MCP could not maintain firmness in fruits stored at chilling temperature unlike those treated fruits stored at ambient temperature.

In previous literatures and our understanding ethylene production in red tomato fruits is reduced under chilling temperature and hence, the concentration of lycopene too. In this experiment, we tried to add external ethylene to compensate the amount of ethylene that would be eliminated by the chilling injury and see if the amount of lycopene to remain same. However, the addition of external ethylene did not result in the maintaining of lycopene content. This will lead us to the assumption that ethylene synthesis might have been suppressed somewhere in the pathway. Therefore, further study is needed to understand which gene(s) is(are) suppressed in the pathway of ethylene synthesis.

CONCLUSION

Finally, the study confirmed that external ethylene application on red tomato fruits stored at chilling temperature generally has no effect on the subsequent accumulation of lycopene or to maintain the loss of accumulated lycopene during chilling storage. However, external ethylene treatment on breaker stage tomatoes helps for red color development. In addition, 1-MCP treatment helps to reduce loss of firmness of the fruits. However, it delays colour development of the fruits, especially in tomatoes stored at non-chilling temperatures.

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