Tomato Fruits (*Solanaceae Lycopersicon esculentum* Mill.) Feedback Mechanism in The Presence of Exogenous Ethylene under Prolonged Chilling Temperature Storage

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Abstract

Green mature and red ripe tomato 'Roma VF' fruits were stored in air-tight glass jars (1000ml), at 5^{o} C for three weeks in dark. Grouped by similarity in color, three fruits were put into each jar. Half numbers of the jars were injected with 2ml of 1.25 ppm Ethylene gas, while the other half was used as control. Four random sets of samples, with three replications, were measured at 7 day intervals within three weeks. Upon measurement, jars were put into 20^{o} C room temperature for 30 minutes, and then the upper atmosphere inside the jar was analyzed using gas chromatogram. The fruits physiology inside each jar were measured (skin and pericarp color and hardness, storing and peeling weight loss, dimension and transversal cross section, soluble solid content, and tritatable acidity). The result showed that red ripe tomato fruits 'Roma VF' stored in 5^{o}C with exogenous ethylene have superior appearance, higher respiration production and higher senescent rate as compared to the control. The green mature fruits had the opposite effect when treated with ethylene, possibly due to an auto-induction mechanism in the synthesis process of the enzymes that are involved in ethylene biosynthesis controlled by the phyto-hormone ethylene. Thus, green mature fruits, when exposed to C2H4, revealed a negative feedback mechanism.

Keywords: Tomato, C2H4, Cold Storage, Feedback Mechanism, Auto-Induction Mechanism.

1. Introduction

Most of the fresh products, including tomatoes, were sent by means of cold-chain (Rodrigue and Notteboom, 2013), to prolong products fresh life, and maintain the fruits in fresh condition at the destination. Recent studies have identified that minimum storage temperature for the tomatoes were around 10 to 15 ^{o}C for red ripe fruits (Saltveit, 1997) and 12 to 20^{o}C for mature-green (Wang, 2004). The problems come when tomato were transported in mix with the other agricultural products, especially certain vegetables that have lower optimum storage temperature (i.e. 5°C) (The Engineering ToolBox, 2014).

This condition may lead tomato to chilling injury (CI), especially when exposed to a prolonged period of chilling temperature (Wang, 2004). In this condition, various physiological and biochemical alterations and cellular dysfunctions occur (Raison and Orr, 1990), and when fruits removed to warmer temperatures, the chilling injuries symptoms will become evident in a short time (Wang 2004), subsequently reduced the fruits quality (Cheng and Shewfelt, 1988), and made it susceptible to decay (Maul et al, 2000). Nevertheless, chilling sensitivity in tomatoes may differentiations according to storage temperature and duration of exposure (Wang, 2004), as well as maturity and degree of ripeness.

Over the years, several methods in alleviating the chilling injury in tomatoes have been tried and have shown positive results (Wang, 2010). These methods include short-term hot (Akbudak and Akbudak, 2007) or cold water treatments (Gharezi et al., 2012), long-term air heat treatments (McDonald et al., 1996; Lurie and Sabehat, 1997) in combination with ethylene treatment (McDonald et al., 1998), intermittent temperature treatment (Artes et al., 1998), dipped in chemical solutions (Gharezi et al., 2012), Controlled Atmospheric storage (Tasdelen and Bayindirli, 1998), as well as other chemical applications or genetic manipulation (Meir et al., 1996).

Nevertheless, these methods require certain preconditions that increase shipping cost and waste considerable time of valuable fresh life of the fruits. In addition, due to the increasing concern for the environment and health, options of waxing or chemical coating to the fruits were limited. On the other hand, ethylene gasification of tomato fruits during storage or shipping show promising future and could be used as one of possible solutions in reducing the CI risks.

The objectives of this study were to investigate the physical changes and respiration effects of industrial tomato cv. Roma VF upon storing with ethylene treatment in controlled atmospheric condition in 5 ℃ temperature, that is optimum for the transport and storage of many fruits and vegetables growth in the Mediterranean, under dark condition, for 3 weeks. The differences in the parameters change; fruit color, weight, dimensions, hardness, soluble solid content, acidity, and its climacteric process (via ripening gases), were observed in 7 days interval until 21 days.

2. Materials and Methods

2.1 Fruits Samples

Tomatoes fruits (cv. Roma VF) with uniform size and mass, harvested under normal field conditions from 2 ripeness categories: red ripe and green ripe (Fig.1) were selected. Complete randomized block design was used to carry out four treatments with three replications. All samples were cleaned and separated into two groups; red ripe and green ripe tomatoes. The first two groups of red ripe and green ripe fruits were used as controls, while the second groups were treated with a modified atmosphere condition (MAC). Initial condition of each fruit was measured and weighed by using an analytical balance (ALS 220-4, KERN & Sohn, Germany), before applying the treatment, which served as its initial weight.

Fig. 1 Tomato sample with different ripeness, 1 and 6 were the two selected ripeness categories representing the green ripe and the red ripe, respectively.

2.2 Physiology Measurements

Fruits color was recorded in three points along its equatorial with 120^o separation distance using Chroma meter (CIE Lab) and expressed as color space L*, a*, b* mode. The color measured in color index as ratio of a* and b* (McDonald et al., 1996; 1998).

Air-tight glass jars containers (1000ml) were prepared to store the fruits. Every jar's cap was drilled to create a 1 mm diameter hole to enable collecting the gas inside the jar for sampling. To avoid gas inside the jar leaked during storage, the hole was re-sealed with transparent silicon. This enables the opening to be easily identified and used to collect gas sample inside the jar by syringe.

All jars were numbered; one from each group will be used to measure the samples in each week for three week storage. Jars of groups 1 and 2 were used for the red ripe control and green ripe control sample, while group 3 and 4 were used for the ethylene treated red ripe and green ripe fruits. Three tomatoes with similar size, weight, and color were put inside each jar. For jars of group 2 and 4, green ripe and red ripe tomato fruits were put inside, and then, each container was injected with 2 ml of 1.25 ppm ethylene gas through the prepared hole on the caps, and resealed with silicon afterward.

During storage, each fruit will be measured to assess its climacteric process by mean of assessing its skin and pericarp; color and hardness development, its weight loss; during storage and after peeled, the dimension of the fruits and its transversal cross section, soluble solid content (SSC), as well as acids level in the fruits using titration. The assessment was done at 7 days interval until 21 days.

2.3 Gas Chromatography Analyses

For the ethylene and respiration measurement of the fruits, all jars were kept in inside refrigerated dark and the temperature was maintained at 5^o C. On zero, 7, 14, and 21 day, three jars from each group was put into 20^o C room temperature for 30 minutes, and then one ml gas of the upper atmosphere inside the jar was drawn with a gas-grade syringe (Gold Syringe, Agilent Technology, USA) and measured using Gas Chromatogram machine (GC system 7890B, Agilent Technology, USA). The result of GC measurement from each sample was compared with the standard (1.25 ppm Ethylene) provided from Agilent. All results were analyzed by quantitative and qualitatively. .

2.4 Physiology Changes Assessment

After the headspace gas inside each container was measured using GC machine, the container was opened, and the tomato fruits inside each container were assessed. The fruits were re-weighed using Analytical balance (ALS 220-4, KERN & Sohn, Germany), to determine its physiological weight loss (Moneruzzaman et al., 2009).

The fruits colors were measured by Chroma meter (CR-400, Minolta, Japan) (Luengwilai and Beckles, 2010). Three points around equator for each fruit was measured in color using CIE Lab standard chromatogram, and average. The same measured points were then peeled to expose its pericarp, and the color was recorded and average. The color index of the fruits skin and its pericarp will be used to compare the effects of the treatment to the samples (Roberts et al., 2002).

A standard peeler was used to peel the fruits. The fruit and its peel were measured to compare the weight loss during peeling process. The hardness of the peel and pericarp from each fruit were measured using penetrometer (FT327, Italy) and the result was recorded in $kg.mm^{-2}$ (Gharezi et al., 2012). The peeled fruits were then cut transversally into two parts along its equator line to assess the internal appearance (equatorial section) of fruits pericarp color and thickness, number of locules and its gel formation, and its septum width (Kader and Morris, 1976). The peeled fruit was then individually liquidated using a mechanic juicer, and its SSC was measured using refract meter (ATC-1, Atago, Japan) (Gharezi et al., 2012).

Total tritatable acidity was measured by taken 5ml of each fruits liquid, and then added with three drops of dye Indicator (phenolphthalein) and measured using automatic titration machine (Tritomatic 25, Crison, Spain) to measure its acidity (Nair and Singh, 2003). The fruits flavor was measured by the ratio of SSC and total titratable acid, calculated by dividing SSC percent with the corresponding acid percent and expressed as a ratio (Baldwin et al., 1998).

2.5 Statistical Analyses

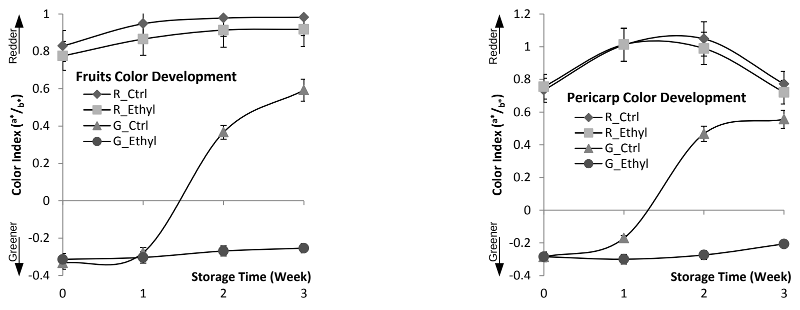
Descriptive statistical analyses by means of Analysis of variance (ANOVA) were used to analyze the differences between treatments and control as well as their variation among and between groups. The data pertaining to physiological and biochemical parameters of the fruit were subjected to statistical analysis using analysis of variance (ANOVA) according to Fischer's. The level of significance used in F test and t test was set at $p \leq 0.05$. Critical difference values were calculated wherever the 'F' test was found significant (Gharezi et al., 2012). The data were subjected to SPSS 20 Statistical Software (IBM Inc., USA).

3. Results and Discussion

The storing of fruits in a lower temperature condition usually suppresses the climacteric and many related activities in fruits, but makes the fruits prone to chilling injury (Yun et al., 2012). Adding exogenous ethylene to the storage room might alleviate chilling stress to the fruits; however, difference in ripeness stage may affect the sensitivity of fruits to the ethylene presence (Atta-Aly et al., 2000). In this study, all of these conditions were set and all of the parameters were measured.

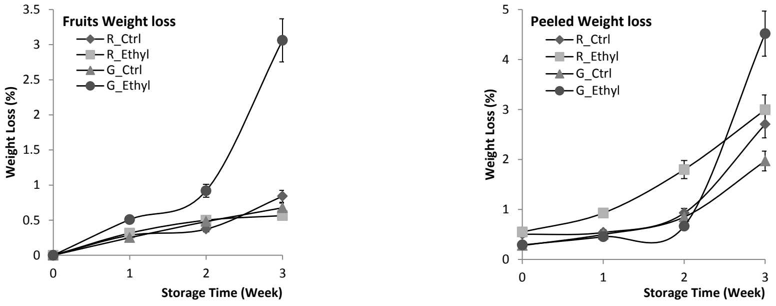
Two groups of tomatoes fruits (c.v. Roma VF) were observed in 7 days interval until 21 days. Stored at 5^{o} C in air sealed transparent containers at dark for three weeks, all physical and chemical change in each group was monitored weekly. The change of color in fruits, as well as its pericarp, can be seen in Fig. 2.

The results indicated that exogenous ethylene treatment did not significantly make difference in color development for red ripe tomato under chilled storage, compared to control. It was observed both in fruits' skin (Fig. 2a) and pericarp (Fig. 2b), even though in general, ethylene treated fruits have slightly less red color as compared to control. Nevertheless, the fruits continue to further develop into fully ripe stage, however, as observed; color degradation was present in the pericarp after the second week of storage. On the other hand, significant difference was showed in green-mature fruits. The presence of exogenous ethylene caused the green-mature fruits unable to further develop into breaker stage (USDA, 1991). The result showed that different ripeness condition of fruits gave different response to their ripening progress when expose to exogenous ethylene in combination with chilling temperature storage. Failed to ripen or de-green was one of indicators of chilling injury (Wang, 2004).



a b

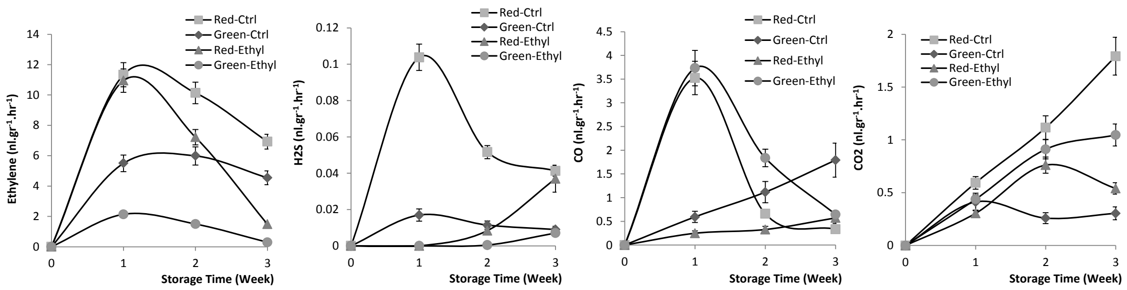
Fig. 2. The color alteration of the samples, measured by the ratio of a* and b* (Color Index) from initial condition until the third week of storage, measured on (a) surface, and (b) the pericarp, for all treatments.



a b

Fig. 3. The samples weigh loss when stored for three weeks (a), and after peeling process (b), for all treatments.

Product loss was another concern upon storing and processing crops and agricultural products for long period. Cold storage has been known to prevent such losses, and while combined with ethylene treatments, the results might give variance according to the products types and conditions. In this study, results showed a positive correlation between storing time and product weight loss as well as loss upon peeling process (Fig. 3). For the red ripe tomatoes, ethylene treatments showed no significant weight loss upon storage compared to control (Fig. 3a), but higher loss observed after peeled (Fig. 3b). On the other hand, green mature fruits were significantly affected by the ethylene treatment. The results observed both in total weight loss (Fig. 3a) and total loss after peeling (Fig. 3b) were significantly different compared to control. The rate of loss increased exponentially after fruits stored for two weeks. The results once again showed that different ripeness stages of fruit significantly affect the result of exogenous ethylene presence in cold storage condition. The results agree with previous research showing that tomatoes were more sensitive to chilling in less mature stage (Paull, 1990).



a b

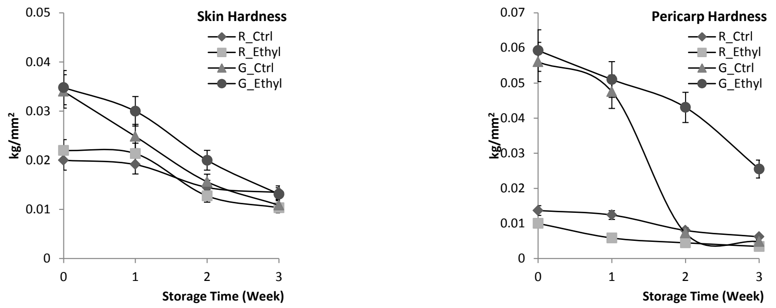
c d

Fig. 4. The climacteric, respiration and decaying process of the fruits, measured by its (a) Ethylene, (b) H2S and (c) CO, and (d) CO2 production rate respectively using GC.

The climacteric process, respiration and decaying rate of the fruits were measured using GC. The climacteric process in fruits measured as fruits' ethylene production rate (Fig. 4a). Fruits decaying rate was determined by the rate of H2S gas produced during storage (Fig. 4b), while the respiration rate of the fruits measured as production rate of CO (Fig. 4c) and CO2 (Fig. 4d). For the red ripe tomatoes, exogenous ethylene addition to the fruits shorten the burst ethylene production period, accelerating the normal process of climacteric, but with combination of low temperature storage, caused reduction in their respiration and senescence rates compare to control. For the green mature tomatoes however, the ethylene addition increased the respiration rate, while their ethylene production from fruits as well as its senescence rates was reduced. This might be corresponding to the fruits condition which failed to enter the breaker stages due to chilling injury. The results (Fig. 4) also showed that the trend of climacteric, respiration and senescence did not significantly correspond to each other, thus suggesting that each process might be triggered by different parts of the fruits, and affected to another different part.

Previous results showed that exposing immature tomato fruit to C2H4 revealed a negative feedback mechanism in relation to its biosynthesis, where ACC concentration and C2H4 production were suppressed, while CO2 production increased significantly (Atta-Aly et al., 2000) as well as CO. However, this condition was vice-versa to red-ripe tomatoes, where ethylene treatment suppresses the CO (Fig. 4c) and CO2 (Fig. 4d) production. The burst of C2H4 (Fig. 4a), H2S (Fig. 4b) and CO (Fig. 4c) were observed in control for the first week, while green mature fruits treated with ethylene showed similar burst only in CO production.

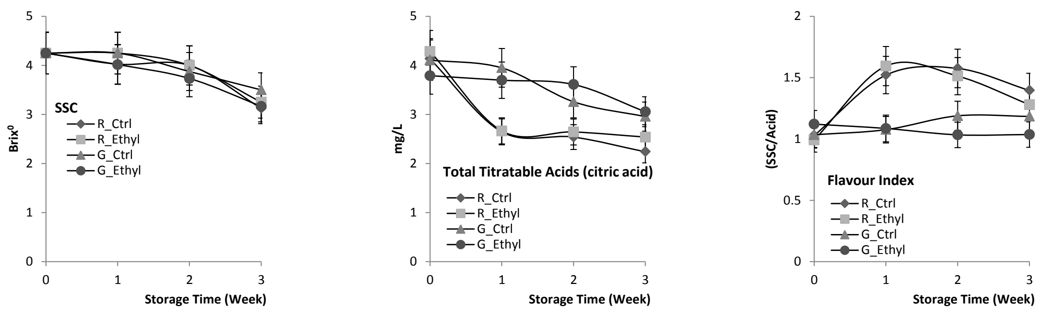
In this work, a negative feedback mechanism in relation to biosynthesis was observed when mature green tomato was exposed to exogenous ethylene, suppress its C2H4 production while multiplying respiration (CO and CO2) production, possibly resulting in lower ACC synthase enzyme concentrations, and catalyzing down-regulation of ripening-associated genes. This may have caused the fruits unable to mature.



a b

Fig. 5. The change of hardness of the samples' peels (a), and pericarp (b), of all treatments measured with fruits penetrometer during storage.

To understand the ethylene treatment effect to fruits peels and pericarp, both parts' firmness were measured. The results (Fig. 5) showed that over the storing time, the hardness of fruits peels and pericarp were reduced. The ethylene treatment in combination with storing tomatoes in low temperature slightly reduced the peeling hardness of the red ripe fruits; while significantly produced harder skin in the green ripe fruits (Fig. 5a). As for the pericarp, only green ripe fruits treated with ethylene showed higher hardness compared to other samples (Fig. 5b). a possibility explanation would that since the de-greening process did not take place in the ethylene treated green ripe fruits, less chemical reaction might transform the chlorophyll in the pericarp to other compounds. And as such, the cells in the pericarp still constitute a much less ripe condition, observed through its color, and thus maintain its strength longer compared to the green ripe fruits which already had de-greening process (green ripe control).



a b

c

Fig. 6. The change of two chemical components indicator of the samples, measured by means of the (a) SSC, and (b) Acidity, in relation to the (c) flavor index, of all treatments during storage.

The chemical changes in the fruits were measured by the change of its soluble solid content (SSC), acidity and the gases produced by the fruits during storage. The SSC and acidity play important role to the taste of the fruits processing product, the correlation between this two properties can be identified as the Brix/Acid index. The results showed that ethylene treatment for red ripe tomatoes did not significantly affect their SSC (Fig. 6a), and only slightly increased the acidity level during treatment (Fig. 6b). On the other hand, the SSC levels of green ripe tomatoes were comparably lower when treated with ethylene (Fig. 6a), while their acidity levels tend to rise over the time of storage, in opposite to the green ripe control (Fig. 6b).

The differences of total SSC and titratable acids between ethylene treated and non-ethylene treated of both red and green ripe tomatoes will lead to differences in taste of the samples after stored for a certain time. Since taste was one of the important factor that dictate the products quality in the tomato processing industry, storing the raw materials before process had to be managed to prevent the negative effects to the product taste, while maximizing its shelf life. Decreasing the storage temperature, storing the products in the dark as well as managing ethylene gas from external sources were ways to maintain the fruits quality and end product taste upon stored for longer period. In this study, the taste measured by comparing the SSC to the acidity of the samples. The results (Fig. 6c) indicated that only the green tomatoes with ethylene treatment have significant lower SSC to acid ratio after the second weeks, while for the red tomatoes, their SSC to acid ratio were considered similar with or without the ethylene treatment. The results indicated that under low temperature storage, fruits maturity determined the change in acidity and taste while ethylene treatment showed less significant influence.

The results in this work showed some significant distinction and benefits in handling industrial tomato fruits (c.v. Roma VF), compared to the previous works.

5. Conclusion

In this study, red ripe tomato fruits 'Roma VF' stored in 5^{o}C with exogenous ethylene had more superior appearance after stored for three weeks, compared to non-ethylene treated fruits. The results also observed differences in the respiration production and senescent rate. On the other hand, green mature fruits had the opposite effect when treated with ethylene. The fruits unable to ripen possibly due to auto-induction mechanism in the synthesis process of the enzymes that involved in ethylene biosynthesis, such as ACC synthase (ACS) enzyme and ACC oxidase (ACO) enzyme. These enzymes controlled by the phyto-hormone ethylene, when exposed to C2H4 revealed a negative feedback mechanism to immature tomato fruit. This is correlated with the condition where less mature fruits were more sensitive to chilling injury, thus greatly influencing its biosynthesis, where ACC concentration and C2H4 production were suppressed, while CO2 and CO production increased significantly.

The results of this work also showed several beneficial outputs, especially upon handling the processing tomato fruits, as compared to different methods in previous works.

Acknowledgments

The first author would like to thank the Panacea project for providing the PhD mobility scholarship under Erasmus Mundus Action 2 program, the Directorate General of Higher Education of Indonesia for providing the PhD fellowship in Asian Institute of Technology, Thailand, and to the LPDP Indonesia, for providing funding for his dissertation work.

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