PHYSIOLOGICAL STUDY ON TOMATO FRUIT TO KEEP FRESHNESS UNDER HIGH-TEMPERATURE CONDITIONS

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ABSTRACT

This paper studied the possibility of high-temperature storage of green mature tomato fruit. First, green mature tomato fruit were stored at 25, 31, 33, 35, and 37 ^{0}C for 17 days and thereafter shifted to 25 ^{0}C to check the effect of storage temperature on fruit freshness before and after shifting to 25 ^{0}C. In another experiment, tomato fruit were harvested at green mature stage and stored at 33 ^{0}C for 0 (control), 5, 10, 20 and 30 days, thereafter shifted to 25 ^{0}C to investigate the effect of high-temperature storage on fruit freshness before and after shifting to 25 ^{0}C. This research was conducted at Tokyo University of Agriculture, Japan from 2017-2019. Fruit color development, weight loss, fruit firmness, ethylene production, CO_2 release, Brix, sugars, organic acids, 1-amino cyclopropane-1-carboxylic acid (ACC) content, and ACC oxidase (ACO) activities were measured as freshness indices. The possibility of longer storage of green mature tomato fruit under high temperature was discussed, which might well contribute to facilitate the harvesting operation in the summer season in developing countries, like Southeast Asian countries.

Key words: Ethylene, firmness, fruit color, green mature tomato, respiration, *Solanum lycopersicum*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important crop in Solanaceae family which has been widely used in both fresh and processed consumption patterns (Harvey et al. 2003), and its production has increased to approximately 182 million tons by 2017 (FAOSTAT 2019). Tomato contributes significant nutritional components to human health (Stommel 2007), and has been documented as foods with potential chemo-preventive activities against several chronic diseases because of the high levels of lycopene and other bioactive compounds (Giovannucci et al. 1995; Giovannucci 1999, 2002). Maturity stage of tomato fruit at harvesting time is one of the important factors for storage life and final fruit quality (Alam et al. 2006). In developing countries, farmers usually harvest tomato fruit at the ripe stage, while it is considered that ripen fruit is easily damaged and resulted in shorter shelf-life (Kader et al. 1978; Reid 2002; Toivonen 2007; Watkins 2006). Since tomato fruit is perishable (Javid Ullah, 2009, and Nasrin et al. 2008), and it contains a large quantity of water (93.5%) (Gastélum et al. 2011), it eventually leads to severe post-harvest losses in some cases. Shelf-life is determined as the time that a product is acceptable and meets the consumers' preference (Martins et al. 2008).

Post-harvest treatments play an important role in extending the storage and marketable life of horticultural perishables (Ramady et al. 2015), but the quality of vegetables and fruits cannot be improved after harvest (Genanew, 2013; ATTRA, 2000; Ramady et al. 2015). The shortage and instability of electricity and lack of proper cold storage are also shelf life limiting factors for several fruits and vegetables in developing countries. In addition, because tomato is cold susceptible, low temperature storage sometimes declines the fruit quality (Kader et al. 1989; Parnell et al. 2004; Soto et al. 2005).

One of the possible solutions for these challenges to minimize tomato fruit damage during transportation and to restart ripening in retail channels is to harvest tomato fruit at green mature stage (Kader et al. 1978; Wang et al. 2008; Reid 2002). In case of industrial production systems, tomato can be harvested mechanically at green mature stage, packed into crates, sorted, sized, washed, cooled, stored and transported over long-distances (Beckles 2012). The storage of green mature tomato fruit at 38 ^{0}C prior to 2 ^{0}C storage could remove chilling injury development for up to 30 days without causing heat injury (Lurie and Sabehat 1997). Two days exposure to 38 ^{0}C allowed green mature tomato fruit to ripen normally without chilling injury symptom after two weeks storage at 2 ^{0}C (McDonald et al. 1998). The storage of green mature tomato fruit at 33 ^{0}C for 5-33 days could prolong its postharvest freshness even when it was shifted to room temperature though he did not show the physiological mechanism (Ogura 1975). Green mature tomato fruit could only be stored for 2-3 days in 40 ^{0}C, but additional information was not provided (Ogura 1976).

Since the limited researchers have investigated post-harvest handling and physiology of green mature tomato fruit under high-temperature storage, this study aims to evaluate the physiological characteristics of green mature tomato fruit to determine the most suitable temperature and duration of high temperature storage. This work might contribute to the harvesting operation in summer season and help the farmers and storekeepers in Southeast Asian countries to increase their income by prolonging fruit freshness so that they could contribute to maintain food security through reduction in post-harvest losses.

MATERIALS AND METHODS

This research was conducted at Tokyo University of Agriculture, Japan, during 2017-2019. Firstly, green mature tomato fruit (RED ORE variety, Kaneko seed company) was harvested in the morning every time at Green Farm Lapin Co. Ltd., Namegawa town, Saitama prefecture, Japan and soon brought to Setagaya Campus of Tokyo University of Agriculture and the uniform fruit were selected by ethylene production with almost same level of emission.

Effect of temperature on the post harvest physiology of green mature tomato fruit.: Green mature tomato fruit was stored at 25, 31, 33, 35, and 37 ^{0}C for 17 days with 10 replications using ETTAS (Environmental Testing Tools by Asone) model IW-300SB, Japan incubators and thereafter shifted to 25 ^{0}C to check the effect of different storage temperatures on the physiological changes as mentioned below and to check whether maturation could restart when shifted to room temperature.

Effect of storage duration under high temperature on the post harvest physiology of green mature tomato fruit: Green mature tomato fruit were stored at 33 ^{0}C (which was found the most suitable temperature in our preliminary study) for 0, 5, 10, 20, and 30 days with 10 replications using ETTAS model IW-300SB Japan incubators, and thereafter shifted to 25 ^{0}C to investigate the effect of duration of high-temperature storage on the physiological changes mentioned below and to check whether maturation could restart when shifted to room temperature.

Analysis of ethylene metabolism of green mature tomato fruit under high temperature storage: Green mature tomato fruit were stored at 25 and 33 ^{0}C for 7 days and ACC contents, ACO activity and ethylene production were analyzed to know the changes in ethylene metabolism.

Fruit color was measured using Handy Colorimeter NR-3000, Japan. Weight loss was calculated for each interval and evaluated as a percentage against initial weight using simple scale HF-4000, and the final weight loss was expressed in the percentage (Tefera et al. 2007). Fruit firmness was evaluated using a Multilateral Tester Model 2519-104 (INSTRON Co. 3342R4200, U. S. A), and the data were indicated as N when 1.0 cm diameter plunger pressed tomato fruit at 1 mm/sec speed.

For ethylene production, each fruit was placed inside a 550 mL glass jar and incubated under dark condition for an hour at room temperature. Ethylene production was analyzed after 1-hour incubation. Head space gas of 1 mL was taken out with a plastic syringe and analyzed by Shimadzu GC-14B equipped with a FID (flame ionization detector). The analysis conditions were injector: 180 °C, column: 80 °C, and detector 200 °C. The column used was Sunpack A (Shinwa Kako, 2.1m×3.2mm ϕ glass column filled with porous polybeads). The carrier gas was N_2, and the column pressure was maintained at 6 kg cm^{-2}. CO_2 was simultaneously analyzed after 1 hour by GC-TCD (Gas Chromatography-Thermal Conductivity Detector, Shimadzu Japan) using the headspace method just as same as ethylene production. The equipped column was Shinwa Kako glass column, 2.1 m×3.2 mm ϕ, filled with porous polybeads) and analysis conditions were injector:150 °C, column: 40 °C, and detector: 150 °C.

Asone refractometer was used to measure Brix value shown in percentage (%). Sugar and organic acid levels were quantified using the method described by Ayvaz et al. (2016), and Vazquez et al. (1993), respectively, with slight modifications. Ethanol of 1.8 mL 50 % was added to 0.2 g crushed powder of tomato fruit using 15 mL falcons and centrifuged for 10 min at 12000 rpm under 4 ^{0}C. The supernatant was filtered with 0.20 µ PTFE plus 0.45 µ Cellulose membrane filters and analyzed by HPLC (High-Performance Liquid Chromatography: Hitachi-Model, D-7000IF, Hitachi, Ltd. Tokyo, Japan). Sugars were analyzed by Shimadzu analyzer, using detector RID-10 A refractive index detector with a flow rate of 0.8 mL/min of deionized water. Column was Shodex sugar KS-801 with 300 mm×8 mm φ in oven temperature 80 °C. Organic acid was analyzed using Shimadzu CDD-10AVP, conductivity detector, equipped with column series SCR-102H×2 (300 mm×2×3.2 mm φ) in the oven set at 40 °C with a flow rate of 0.8 mL/min of 20 mM p-toluene sulfonic acid.

For the ACC measurement, 2 g crushed and frozen tomato fruit tissues were weighed in a 15 mL falcon tube and cooled in liquid nitrogen. Then 4 mL of 5 % SSA (Sulfosalicylic acid) solution was added, and vortexed until a homogeneous mixture was obtained. The samples were extracted for 30 min while gently shaked at 4 °C and centrifuged for 10 min at 3,090 × g in a precooled centrifuge at 4 °C to get the supernatant. Two sets of analysis were prepared; one for without standard d-labeled ACC and the other with known amount of d-labeled standard ACC to check the recovery rate of extraction. Then immediately the samples of the headspace were taken to GC-MS for the ACC content analysis. The analysis conditions of GC-MS (GC-MS-QP2010 plus SHIMADZU) and the column used was DB-5 as 0.25 mm of the internal diameter, 300 mm of length and 1.00 µm of thick film (Agilent Technologies Inc). GC condition was as follows; the inlet temperature was set at 280 °C, injection was split 10:1, the oven temperature was set detained for 1 min at 60 °C and raised to 320 °C at a rate of 4 °C/min and kept 10 min. He gas flow rate was 1.1 mL/ min. The scan mode analysis was adopted by MS, and the transfer line and ion source were set at 290 °C and 200 °C, respectively. The mass spectra were recorded at a scan/s with m/z 45-600 scanning range.

To measure ACO activity, 50 mg of polyvinylpolypyrrolidone (PVPP) were weighed in a 2 mL microcentrifuge tube. Crushed tomato fruit tissues of 500 mg were added after cooling the microcentrifuge tube in liquid nitrogen. Then 1 mL of extraction buffer was added. After the homogeneous mixture was obtained by vortex, the sample was incubated in a thermomixer for 10 min at 4 °C, with gently shaking. Centrifuged for 30 min at 22,000 × g in a pre-cooled centrifuge at 4 °C, the pellet was discarded, and the supernatant was collected. The microcentrifuge tubes were kept on ice, and the reading has taken place immediately after the addition of 10mM ACC solution.

The samples for headspace were analyzed by GC for ethylene content. The analysis conditions were the same as the ethylene measurement mentioned above.

Statistical analyses were conducted using SPSS statistical software (SPSS 16.0, Prentice-Hall, New Jersey, NJ, USA) and the significant differences were determined at the $P < 0.05$ probability level. Means were compared using the Tukey's HSD test.

RESULTS AND DISCUSSION

As for the effect of storage temperature on tomato fruit freshness, storage at 25 ^{0}C (control) developed deep redness, while treatment of higher temperatures showed slow pigmentation, especially in higher temperatures as 31, 33, and 35 ^{0}C (Fig. 1, left panel and Fig. 3). If the fruits are transferred to room temperature, tomato fruit could achieve maturation even after 30 days storage under high temperatures (Fig.1, right panel). Because the higher temperature provided more yellowish skin color, the process of red and yellow pigmentation seems to be regulated separately by temperature dependent way. It was reported that high temperature stress inhibited lycopene synthesis, and the heated tomato could restart color development after the removal of temperature stress (Cheng et al. 1988; Lurie and Klein 1992). It has been observed that keeping green mature tomatoes at temperatures above 30 °C frequently become yellowish instead of red color, and the same phenomenon was observed at low temperature below 12 °C (Tijskens and Evelo 1994). It means that red pigment formation is rather susceptible for temperature stress in comparison with yellow pigment.

Fig. 1. The effect of high-temperature storage on pigmentation (redness) of green mature tomato fruit.

Tomato fruits harvested at green mature stage were stored in 25 ^{0}C, 29 ^{0}C: B, 31 ^{0}C: C, 33 ^{0}C: D, and 35 ^{0}C for 17 days (left) and shifted to 25 ^{0}C till 40 days (right). The picture was taken on 17th day of initial storage under each temperature (right) and 40th day including 17 days storage under each temperature and 23 days at 25 ^{0}C. Even though the skin color looks greener under high temperature storage (left), the maturation process could restart when shifted to 25 ^{0}C.

Storage under 33 ^{0}C could exhibit longer freshness only when tomato fruit was stored longer than 20 days (Fig. 2). It is noteworthy that tomato fruit could restart maturation when shifted to room temperature even after 30 days. It means that we could harvest or transport green mature tomato and store under higher temperature more than a month to manage shipping timing.

Fig. 2. Effect of storage duration under 33 ^{0}C on the maturation of green mature tomato after shifting to 25 ^{0}C.

From left; control (0 days), 5 days, 10 days, 20 days, and 30 days. The photo was taken on 60th day after initial storage.

It is noteworthy that 30 days storage under 33 ^{0}C can provide the longer freshness when shifted to 25 ^{0}C judged from skin appearance.

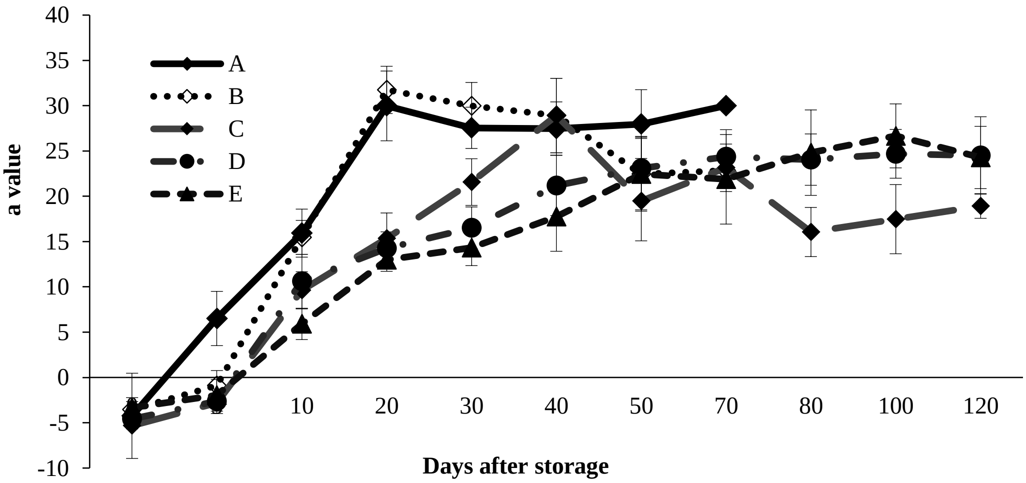

Fig. 3. Effect of high-temperature storage on color (redness) of green mature tomato fruit.
Tomato fruit was harvested at the green mature stage and stored at 33 ^{0}C and thereafter shifted to 25 ^{0}C. As 0 day: A control, 5 days: B, 10 days: C, 20 days: D, and 30 days: E. Data are indicated in the means of 10 replications with SE.

The weight loss of tomato fruit was prevented most at 25 ^{0}C, and the longer the storage at 33 ^{0}C, the more the weight loss (Fig. 4). It might be influenced by the less transpiration from skin surface at 25 ^{0}C.

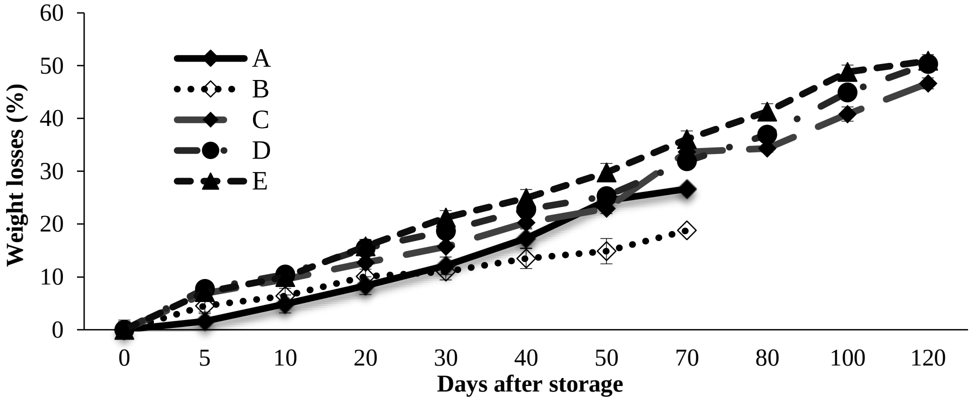

Fig. 4. Effect of high-temperature storage on weight loss of green mature tomato fruit.

Tomato fruit was harvested at the green mature stage and stored at 33 ^{0}C and thereafter shifted to 25 ^{0}C. As 0 day: A control, 5 days: B, 10 days: C, 20 days: D, 30 days: E. Data are indicated in the means of 10 replications with SE.

Fruit firmness decreased faster when stored at higher temperatures, but it recovered little by little after 1-month storage (Fig. 5). It is suggested that fruit peel became harder after losing some amount of water, while after 70-days of storage all treatments gradually decreased their firmness with no recovery.

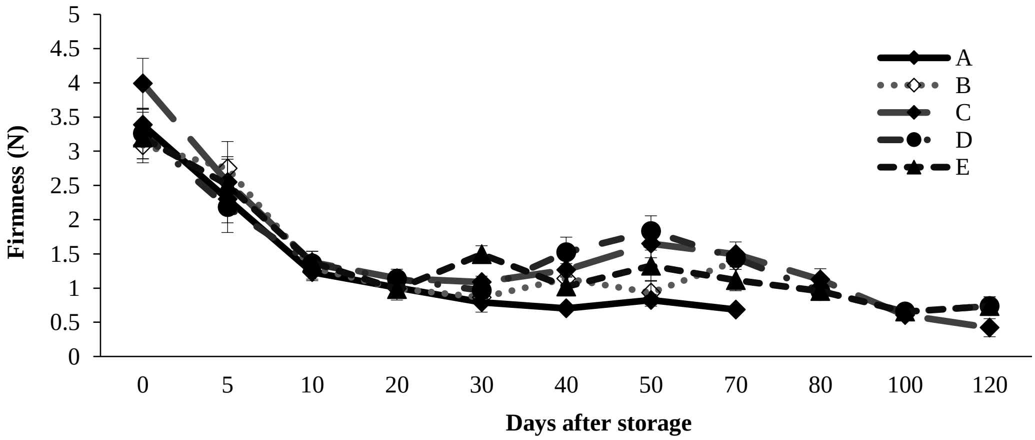

Fig. 5. Effect of high-temperature storage on the firmness of green mature tomato fruit.
Tomato fruit was harvested at the green mature stage and stored at 33 ^{0}C and thereafter shifted to 25 ^{0}C. As 0 day: A control, 5 days: B, 10 days: C, 20 days: D, 30 days: E. Data are indicated in the means of 10 replications with SE.

Tomato is a climacteric fruit and its ripening depends highly on respiration and ethylene action (Yang et al. 1984), so ethylene production during storage is one of the most important indices of post-harvest physiology of tomato fruit. Experimental results show that ethylene production was inhibited by storage under 33 ^{0}C for different durations in comparison with 25 ^{0}C, and it was kept low even after shifting to 25 ^{0}C (Fig. 6). The ethylene production was suppressed at higher temperature, but whenever shifted to 25 ^{0}C, it rapidly increased in case of 5 days storage at 33 ^{0}C (Fig. 6); it increased from 2 nL $g^{-1} h^{-1}$ to 9 nL $g^{-1} h^{-1}$ within 5 days. Similar rapid increase was also observed after 10 and 20-days storage at higher temperature, while after 40 days it couldn't show any further increase in ethylene production. Ethylene production was inhibited by the storage under 31, 33, 35, and 37 ^{0}C in comparison with 25 ^{0}C, it was also kept low even after shifting to 25 ^{0}C after 17 days (data were not shown). The results were consistent with Lurie et al. (1996) and the critical turning point seems to exist lower than 31 ^{0}C in case of fruit ethylene production. After 30-days storage in higher temperatures, all treatments were found to inhibit ethylene production.

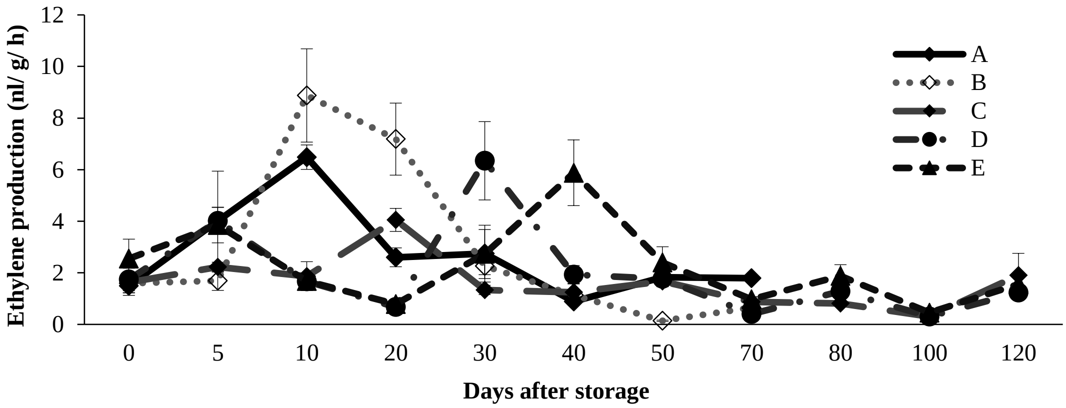

Fig. 6. Effect of high-temperature storage on ethylene production of green mature tomato fruit. Tomato fruit was harvested at the green mature stage and stored at 33 ^{0}C and thereafter shifted to 25 ^{0}C as 0 days: A control, 5 days: B, 10 days: C, 20 days: D, 30 days: E. Data are indicated in the means of 10 replications with SE.

The result of respiration was similar to the findings of Ogura et al. (1976), in 25 ^{0}C as well as after treating with 33 °C, it resulted in the suppression of respiration in tomato fruit (Fig. 7.) This action was not completely reversible after shifted to 25 ^{0}C or ambient temperatures. The results of this study showed that at high temperatures, respiration rate decreased at longer storage duration (Cheng et al. 1988; Inaba and Chachin 1989; Lurie and Klein 1991).

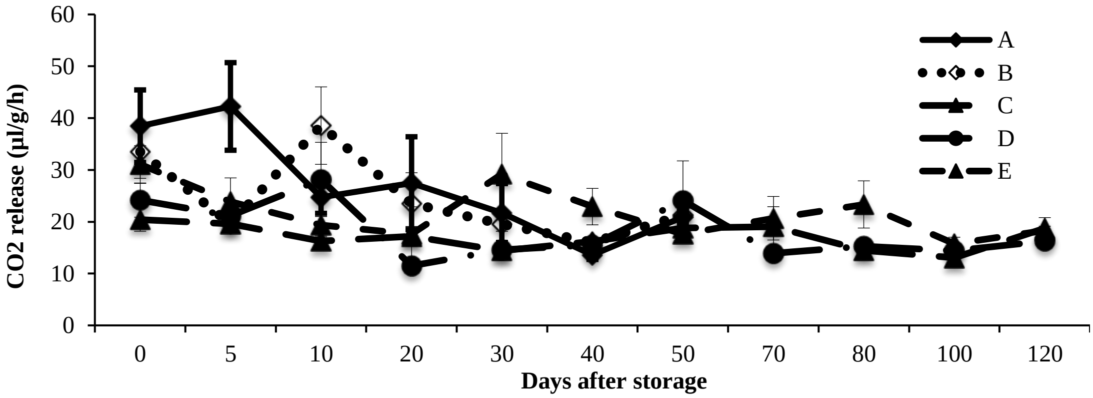

Fig. 7. Effect of high-temperature storage on the CO_2 release of green mature tomato fruit. Tomato fruit was harvested at the green mature stage and stored at 33 ^{0}C and thereafter shifted to 25 ^{0}C as 0 days: A control, 5 days: B, 10 days: C, 20 days: D, 30 days: E. Data are indicated in the means of 10 replications with SE.

Under 33 ^{0}C, ethylene production was inhibited with the decrease in ACC content as well as the deactivation of ACO (Fig. 8).

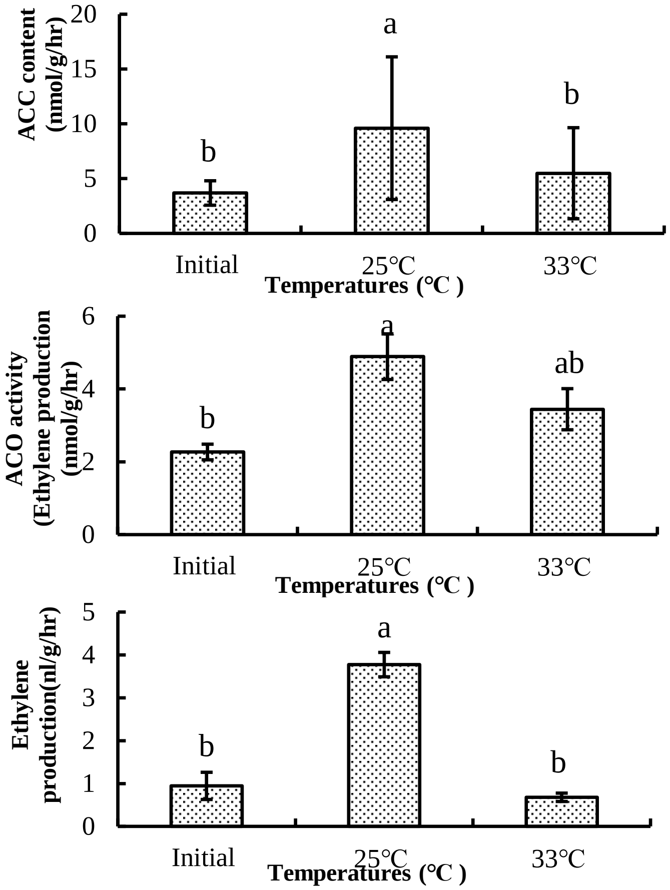

Fig. 8. Effect of high-temperature storage on ACC content, ACO activity and ethylene production of green mature tomato fruit. Tomato fruit was harvested at the green mature stage and stored at 25 ^{0}C or 33 ^{0}C for 7 days. Data are indicated in the means of 3 replications with SE.

The Brix value of the fruit stored in 33, 35, and 37 ^{0}C were slightly higher than those in 25 ^{0}C and 31 ^{0}C, and as the temperature got higher, the Brix value became higher too. It may be due to the concentration effect; higher relative sweetness led by water loss. Since the Brix value of fully ripen RED ORE intact fruit sometimes reaches to as high as 7.0 percent, the values in this experiment cannot be evaluated very low, taking it into consideration that green mature fruit was stored (Fig. 9).

There was a significant difference among the treatments in case of sugars as glucose, fructose, and sucrose. It was observed that higher temperature increased glucose accumulation in tomato fruit until 33 ^{0}C and above that temperature, the glucose contents were decreased. Accumulation of fructose and sucrose were gradually decreased in high temperatures comparing to 25 ^{0}C. The content of organic acids (malic acid and citric acid) were increased at 33 ^{0}C and 35 ^{0}C storage temperatures comparing to 31 ^{0}C (Table 1). Even though malic acid showed insignificant difference among the treatments, it was gradually increased till 35 ^{0}C, while citric acid was high at 33 ^{0}C followed by 35 ^{0}C and 37 ^{0}C.

Table 1. Effect of high-temperature on sugars and organic acids of green mature tomato fruits.

Treatments	**Sugars (g/100g)**			**Organic acids (g/100g)**	
	Glucose	**Fructose**	**Sucrose**	**Malic acid**	**Citric acid**
25 ℃	1.36±0.08 ab	2.02±0.07 a	0.02±0.00 a	0.084±0.02 NS	0.031±0.01 b
31 ℃	1.53±0.04 a	2.11±0.08 a	0.01±0.00 b	0.075±0.00 NS	0.087±0.00 a
33 ℃	1.55±0.08 a	2.01±0.13 a	0.01±0.00 b	0.079±0.01 NS	0.101±0.01 a
35 ℃	1.40±0.13 ab	1.75±0.18 ab	0.01±0.00 b	0.081±0.00 NS	0.093±0.01 a
37 ℃	1.07±0.18 b	1.35±0.22 b	0.01±0.00 b	0.078±0.01 NS	0.082±0.01 a

Tomato fruit was harvested at the green mature stage and stored at 25, 31, 33, 35, and 37 ^{0}C for 17 days and thereafter shifted to 25 ^{0}C. Data are indicated as the means of 3 replications with SE. Different letters show a significant difference at the 5 % level by Fisher' s LSD and NS refers to Non-Significance. A is control treatment or 25 ^{0}C, B; 31 ^{0}C, C; 33 ^{0}C, D; 35 ^{0}C, and E shows the treatment that fruit was stored in 37 ^{0}C.

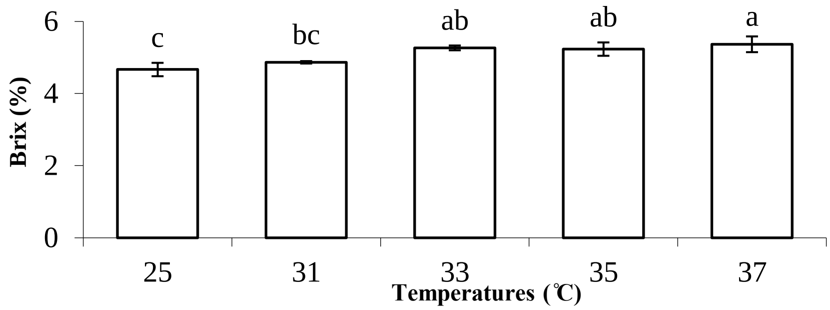

Fig. 9. Effect of high-temperature storage on Brix of green mature tomato fruit. Tomato fruit was harvested at the green mature stage and stored at 25, 31, 33, 35, and 37 ^{0}C for 17 days and thereafter shifted to 25 ^{0}C. Brix was measured by pocket refractometer. Data are indicated in the means of 3 replications with SE.

CONCLUSION

It would be best if tomato fruit are pre-cooled just after harvest and transported through cold chain system, but in the case of developing countries of Southeast Asian countries, it is better to consider the alternative way to develop an appropriate method of keeping vegetable freshness without expensive facilities. Based on the above results, we suggest harvesting tomato fruit at mature green stage to provide enough time for transportation to a long-distance market, while it is better to harvest at the fully ripe stage to maximize nutritional value in case of local markets. Some of the practical advantages of green mature tomato fruit storage under higher temperatures were indicated, though more detailed researches might be required to investigate the possible precise metabolic changes. This work might contribute to the harvesting operation in summer season and help enhance the income of storekeepers and ensure food security through reduction in post-harvest losses.

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