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Physiological and Biochemical Effects of Intermittent Warming on 'Marmandi' Turning Tomato Fruits During Low-Temperature Storage

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ABSTRACT

This experiment was conducted on the variety 'Marmandi' turning tomato fruits to investigate the effect of intermittent warming temperatures during extended low chilling temperature storage (21 days) on the subsequent ripening quality of tomato fruits. The quality was measured by assessing the quantitative amount of total phenolic compounds, chlorogenic acid, and ascorbic acid, as well as the development of fruit color and the evaluation of chilling injury (CI) symptoms. The results indicated that the amounts of total phenolic compounds and chlorogenic acid increased significantly during continuous low-temperature storage and after subsequent transfer to room temperature, with this increase being associated with significant loss of ascorbic acid. More frequent periods of intermittent warming (IW) during low-temperature storage were very effective in significantly decreasing the rate of accumulation of phenolic compounds, chlorogenic acid, and ascorbic acid losses. A significant reciprocal relationship between the amounts of chlorogenic acid and ascorbic acid was observed. Additionally, the results showed that as IW time increased, its effectiveness in retarding CI significantly improved.

التأثيرات الفسيولوجية والكيميائية الحيوية للتدفئة المتقطعة على ثمار الطماطم 'مارماندي' في مرحلة التحول أثناء التخزين في درجات حرارة منخفضة

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الكلمات المفتاحية:
ثمار الطماطم
أضرار البرودة
الإحترار المتقطع
المواد الفينولية
حمض الاسكوربيك

الملخص

أجريت الدراسة على ثمار الطماطم *Lycopersicon esculentum* Mill صنف مرماندى في مرحلة النضج المتحول (turning stage) بهدف التقليل من حدة التضرر الفسيولوجي لأضرار البرودة (CI) لثمار الطماطم المخزنة عند درجة حرارة (5° C) بتعريض الثمار لدرجة حرارة الغرفة (الأحترار المتقطع) على فترات مختلفة (1, 2, 3أيام) طيلة فترة التخزين عند درجة حرارة 5° C لمدة 21 يوم. تم دراسة التغيرات في بعض الصفات الطبيعية والكيميائية المتمثلة في تقدير كمية المواد الفينولية الكلية، حمض الكلوروجينيك وحمض الاسكوربيك (فيتامين ج) بالإضافة الى دراسة بعض الصفات الظاهرية والمتضمنة تحديد صلابة الثمار، حدة الإصابة بأضرار البرودة ومعدل تطور لون والنضج في الثمار. تشير النتائج انه خلال وبعد التخزين عند درجة حرارة (5° C) وأيضا بعد نقل الثمار الى درجة حرارة الغرفة لمدة ثلاثة ايام ازداد معنويا معدل تراكم المواد الفينولية، حمض الكلوروجينيك صاحبه فقد معنوي في حمض الاسكوربيك كما تشير النتائج الى انه كلما زاد معدل تعرض الثمار للأحترار المتقطع خلال التخزين المنخفض أدى الى تقليل معنوي لحدوث اضرار البرودة وتقليل نسبة الفقد في صلابة الثمار وازدياد وتطور لون الثمار أيضا لوحظ أيضا ان ازدياد الأحترار المتقطع خلال

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التخزين المبرد قلل من معدل تراكم المواد الفينولية، حمض الكلوروجينك ومعدل الفقد في حمض الاسكوربيك. وبدراسة التغير في بعض المركبات وجد ان هناك علاقة عكسية بين كمية حمض الكلورجينيك وحمض الاسكوربيك. اما التخزين المتواصل بدون أحترار متقطع لمدة 21 يوم أدى الى زيادة اضرار البرودة وقد لوحظ فرق ظاهري كبير بين الثمار المخزنة عند درجة حرارة 5°C وتلك المخزنة في درجة حرارة الغرفة.

1. Introduction

Ripening is the final stage of the developmental program in fleshy fruits. During this phase, fruits become edible and acquire their distinctive flavor and post-harvest potential [1]. Tomato (*Lycopersicon esculentum*) is considered one of the most widely consumed fresh vegetables in the industrialized world [1; 2]. Tomato plants are cultivated for their edible fruits, which are a rich source of human nutrition and a repository of antioxidants such as lycopene and ascorbic acid. These antioxidants help remove reactive oxygen species (ROS) molecules. Many studies [2; 3; 4; 5] have indicated that low temperatures might disrupt the respiratory chain and trigger the production of significant amounts of ROS. Low temperatures also reduce the scavenging efficacy of enzymes and impair antioxidant turnover [3; 6]. ROS are dangerous molecules because they can induce cell death [7; 8].

Ascorbic acid (vitamin C) plays a crucial role in the absorption of iron from other sources [2] and is essential for the formation of normal teeth and bones. An increase in ascorbic acid content in fruits indicates that the fruit is still ripening, while a decline suggests that the fruit is in the senescent stage [2]. The loss of ascorbic acid in cold-stored tomatoes was significantly slower than in air-stored tomatoes [2; 9]. Many studies have shown that treatments aimed at reducing chilling injury (CI) symptoms during cold storage effectively control the development of physiological disorders, improve quality, and delay aging or ripening. This improvement is often accompanied by higher levels of ascorbic acid compared to untreated fruits [2; 8; 10]. Low-temperature storage is generally an effective method for extending the post-harvest life of fruits and vegetables. However, it may lead to substantial economic losses during storage and marketing of sensitive crops [9]. Storing tomato fruits at low temperatures for extended periods is currently risky due to the potential development of CI symptoms [9; 10; 11]. Chilling injury, also known as cold injury, low-temperature injury, or low-temperature breakdown [10; 12], can occur during post-harvest handling, transportation, storage, or even before harvest [8; 9; 12].

Tomato fruits exhibit several symptoms of CI, such as failure to ripen as expected [1;11;13], increased susceptibility to decay by organisms usually not present in healthy tissue [9;13], water-soaking of tissue, and failure to retain firmness [8;11], which reduces shelf life [1;11]. Phenolic compound levels have been reported to increase under low temperatures in chilled tomato fruits [2; 10]. Chlorogenic acid, caffeic acid, and tyrosine have been identified as the main phenolic compounds in some injured crops [10]. The activity of phenylalanine ammonia-lyase (PAL) increased in injured tomatoes [8; 10]. However, CI, which causes serious physiological and malfunction issues, may be mitigated using various techniques. Many studies suggest that intermittent warming (IW) temperatures reduce CI symptoms—similar to other treatments—in tomato [10; 11] and peach fruits [14]. IW may help synthesize new compounds during the warming period and increase the concentrations of polyamines, which can stabilize membranes [15]. Polyamines have antioxidant properties that protect cell membranes against lipid peroxidation, a major symptom of most abiotic stress [11; 16]. Additionally, the accumulation of toxic compounds such as pyruvate, acetaldehyde, and ethanol at low temperatures, which induce CI, can be restored or utilized during warming periods, thereby helping the tissue escape CI [10].

2. Materials and Methods

Marmandi tomato fruits were harvested at the turning stage. The fruits were washed with tap water and air-dried. They were divided into five lots (72 fruits per lot). Each lot contained three samples, with each sample replicated three times. The lots corresponded to the following treatments:

T1: Storage at low temperature (5°C) continuously for 21 days.

T2: Interruption of the chilling period with a 24-hour interval at room temperature after 10 days of storage at low temperature.

T3: Interruption of the chilling period with two 24-hour intervals at room temperature, after 7 and 14 days of storage at low temperature.

T4: Interruption of the chilling period with three 24-hour intervals at room temperature, namely on days 4, 9, and 14 after low-temperature storage.

T5: Storage at room temperature (20-22°C) continuously for 21 days.

After 21 days of storage, all samples were transferred to room temperature for 3 days for subsequent ripening and CI evaluation. For all storage treatments, the relative humidity (R.H.) was fixed at ~75-85%. Fruits were analyzed for total phenolic compounds, chlorogenic acid, ascorbic acid, and firmness at the beginning of the experiment, after the storage period (21 days), and after 3 days of transferring the samples to room temperature. The percentage of fruit color development was recorded twice: after the storage period (21 days) and after 3 days at room temperature. CI was evaluated only at the end of the study, after displaying the fruits for 3 days at room temperature.

Total phenolic compounds were determined according to the procedure described in [10] and expressed as mg/100 grams of tomato fruit. Chlorogenic acid was extracted and determined according to the procedure in [10] and expressed as mg/100 ml of tomato fruit juice. Ascorbic acid was extracted and determined according to the procedure in [10] and expressed as mg/100 grams of tomato fruit. Color development was observed and evaluated using the color chart shown in (Fig. 1) [10; 18]. CI was evaluated visually at the end of the study and expressed as the percentage of total injured fruits.

The statistical design employed was a split-plot with 3 intervals of analysis as the main plot and 5 sub-plots of treatments with 3 replications each. Data were analyzed statistically and tested for significance using Duncan's multiple range test as described by [17].

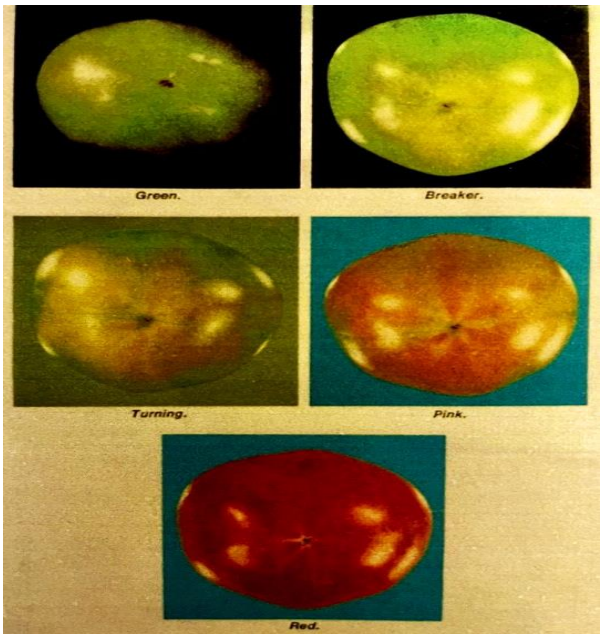

Fig. 1: Stages of tomato fruit ripening as shown in [10; 18]

3. Results and Discussion

3.1 Total Phenolic Compounds

The initial content of total phenolic compounds in turning tomato fruits was 1.74 mg/100 g. This content increased significantly in all chilled fruits, especially in treatments T1 and T2 (Table 1). Similar results were observed after transferring the fruits to room temperature

for 3 days. However, treatments T3 and T4 exhibited significantly lower rates of total phenolic compound accumulation compared to T1 and T2. The data also indicated that T1 and T2 had the highest accumulation rates at the final analysis after 3 days at room temperature, while fruits stored continuously at room temperature (T5) showed a significant decline in total phenolic compounds compared to all chilled treatments.

These findings suggest that treatments involving more frequent warming periods (intermittent warming, IW) during low-temperature storage effectively reduce the accumulation rate of total phenolic compounds. Many studies have demonstrated that IW can facilitate the synthesis of new compounds during warming periods, which can stabilize membranes against lipid peroxidation, a major symptom of abiotic stress [15; 16]. Polyamines, which have antioxidant properties, can protect cell membranes from lipid peroxidation [8; 16]. Additionally, the accumulation of toxic compounds, including phenolic compounds, at low temperatures—which induces chilling injury (CI)—can be restored or utilized during warming periods, allowing the tissue to escape CI [11]. Similar results have been reported in tomato fruits [10] and potato tubers [19]. Increased phenolic compound levels have been linked to the activation of substrates and enzymes under low-temperature storage, such as caffeic acid, chlorogenic acid, shikimic acid, phenylalanine ammonia-lyase (PAL), and tyrosine [10;19].

Table 1: Effect of intermittent warming temperatures on total phenolic compounds in marmandi tomato fruits.

Treatments	Total phenolic compounds (mg/100 g tomato fruits)		
	Initial Content	After 21 days After 3 days at RT*	
Fruits stored at 5°c continuously (T1)	1.74^{H}	9.47^{b}	13.97^{a}
Interruption by one day at RT (T2)		9.37^{b}	14.14^{a}
Interruption by two days at RT (T3)		3.84^{f}	7.47^{c}
Interruption by three days at RT (T4)		6.01^{e}	6.96^{c}
Fruits stored at RT continuously (control) (T5)		2.89^{g}	2.19^{H}

Mean values followed by similar letters are not significantly different at the 5% level according to Duncan's multiple range test.

*Room temperature

3.2Chlorogenic acid and Ascorbic acid

The initial content of chlorogenic acid was 0.97 mg/100 g. Fruits subjected to low-temperature storage showed a significant increase in chlorogenic acid accumulation (Table 2) compared to unchilled fruits, with the highest contents reaching 8.3 mg/100 g in treatment T1 and 7.3 mg/100 g in treatment T2. After transferring the fruits to room temperature for 3 days, chlorogenic acid continued to accumulate significantly in all chilled treatments, while T5 remained relatively unchanged. The minimum accumulation was observed in treatment T4. This indicates that frequent warming during intermittent warming (IW) storage effectively reduced the rate of chlorogenic acid accumulation. Similar results have been reported for tomato fruits [10].

Elmalki (1988) suggested that enzymes involved in chlorogenic acid metabolism, namely hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase and phenylalanine ammonia-lyase (PAL), significantly increase at low temperatures in tomato fruits. This increase in chlorogenic acid levels during chilling is believed to impair mitochondrial activity [20]. However, many studies have indicated that increases in chlorogenic acid levels can be mitigated by storing at temperatures above the critical level.

Table 2: Effect of intermittent warming temperatures on chlorogenic acid content in marmandi tomato fruits.

Treatments	Chlorogenic acid (mg /100 g tomato fruits)		
	Initial content	After 21 days After 3 days at RT*	
Fruits stored at 5°c continuously (T1)	0.97^{i}	7.30^{c}	11.50^{a}
Interruption by one day at RT (T2)		8.30^{b}	11.47^{a}
Interruption by two days at RT (T3)		3.20^{g}	6.15^{d}
Interruption by three days at RT (T4)		4.40^{f}	5.08^{e}
Fruits stored at RT continuously (control) (T5)		1.29^{H}	1.19^{Hi}

Mean values followed by similar letters are not significantly different at the 5% level according to Duncan's multiple range test.

**Room temperature*

The initial amount of ascorbic acid in turning tomato fruits was 15.9 mg/100 g fresh weight. Fruits stored at room temperature exhibited a marked decrease in ascorbic acid levels, dropping to 4.2 mg/100 g. In contrast, chilled fruits showed a highly significant increase in ascorbic acid, with the highest level recorded at 21.4 mg/100 g in treatment T4 (Table 3). No significant differences in ascorbic acid accumulation were observed among the other chilling treatments. After exposure to room temperature for 3 days, all chilled tomato fruits exhibited a significant decline in ascorbic acid content, except for treatments T3 and T4. The most considerable decline was observed in treatment T1. This pattern was also reported by previous studies [10].

Table 3: Effect of intermittent warming temperatures on ascorbic acid in marmandi tomato fruits.

Treatments	Ascorbic acid (mg /100 ml fruit juice)		
	Initial content	After 21 days After 3 days at RT*	
Fruits stored at 5°c continuously (T1)	15.90^{d}	20.46^{ab}	13.33^{e}
Interruption by one day at RT (T2)		19.64^{ab}	16.24^{cd}
Interruption by two days at RT (T3)		18.61^{bc}	16.79^{cd}
Interruption by three days at RT (T4)		21.42^{a}	19.74^{ab}
Fruits stored at RT continuously (control) (T5)		4.30^{j}	4.21^{j}

Mean values followed by similar letters are not significantly different at the 5% level according to Duncan's multiple range test.

**Room temperature*

Chlorogenic acid and ascorbic acid content exhibited a reciprocal relationship. In the chilling treatments (T1 and T2), the increase in chlorogenic acid concentration was accompanied by a reduction in ascorbic acid levels. For instance, when chlorogenic acid reached a maximum level of 11.5 mg in T1, ascorbic acid dropped to a minimum of 13.5 mg. In contrast, treatments with increased interruption periods of warming temperatures (T3 and T4) showed a lesser reciprocal relationship (Fig. 2a and b). Similar observations were made in sweet potatoes, where ascorbic acid was suggested to influence the utilization of chlorogenic acid due to its ability to directly reduce the enzymes responsible for chlorogenic acid metabolism. Therefore, the decline in ascorbic acid may interfere with the utilization of chlorogenic acid, a phenolase substrate. This could explain the observed reciprocal relationship in the concentrations of these substances during chilling.

3.3 Fruit Firmness

Fruit firmness significantly declined in treatments T1 and T2 but showed only a slight, non-significant increase in T4 after the storage period and at the end of the experiment. These results suggest that intermittent warming temperatures were more effective in slowing the rate of decline in fruit firmness (Table 4), likely due to a reduction in chilling injury (CI) symptoms and decay—particularly with longer frequent warming periods—compared to low or room temperature storage.

Table 4: Effect of intermittent warming temperatures on fruit firmness of marmandi tomato fruits.

Treatments	Initial content	After 21 days After 3 days at RT*	
Fruits stored at 5°c continuously (T1)	4.45^{a}	4.04^{ab}	2.10^{defg}
Interruption by one day (T2)		3.11^{be}	2.37^{cfg}
Interruption by two days (T3)		3.33^{abcd}	2.61^{cf}
Interruption by three days (T4)		3.47^{abcd}	3.52^{abc}
Fruits stored at room temperature continuously (control) (T5)		1.29^{g}	1.13^{g}

Mean values followed by similar letters are not significantly different at the 5% level according to Duncan's multiple range test.

**Room temperature*

3.4 Chilling Injury Symptoms

Fruits stored continuously at 5°C for 21 days exhibited severe chilling injury (CI) symptoms, with an incidence rate of 100%. In contrast, intermittent warming (IW) storage temperatures were

highly effective in reducing CI. It was observed that as the frequency of warm temperature periods increased, the effectiveness of reducing CI improved, decreasing the incidence from 100% to 41.6%. This highlights the potential of using alternate storage temperatures to mitigate CI (Table 5). Similar results have been reported by previous studies [11].

Table 5: Effect of intermittent warming temperatures on CI evaluation of marmandi tomato fruits.

Treatments	% CI evaluation
Storage at 5°c continuously (T1)	100[a]
Interruption by one day at RT* (T2)	87.50[b]
Interruption by two days at RT (T3)	83.33[b]
Interruption by three days at RT (T4)	41.66[c]
Storage at room temperature continuously (control) (T5)	†

Mean values in columns followed by similar letters are not significantly different at the 5%
Level according to Duncan's multiple range test
*RT (Room temperature)
† (Not included in the evolution)

3.5 Fruit Color Development

Tomato fruits stored at room temperature became overripe and reached the senescent stage. In contrast, fruit color development from the turning stage to the fully mature red stage was significantly suppressed after prolonged exposure to chilling temperatures (T1) (Table 6). However, intermittent warming (IW) treatments had a positive effect on color development, with increased effectiveness as the frequency of high-temperature breaks increased. Specifically, treatment T4 showed notable improvements in color development, with percentages changing from 0.0%, 25%, and 75% (for turning, pink, and red stages, respectively) to 0.0%, 20.8%, and 79.16% after subsequent transfer to room temperature for 3 days. These results are consistent with findings from previous studies [11].

Although fruits stored continuously at room temperature achieved the highest rate of color development (all fruits reached the red stage), their quality was compromised due to softness, wrinkling, and poor acceptability resulting from over-ripening.

Table 6: Effect of intermittent warming temperatures on percentage of development of tomato fruit color.

Treatments	% fruit color development After 21 days at low temp.			After 3 days at RT*		
	Turning	Pink	Red	Turning	Red	Pink
Fruits stored at 5°c continuously (T1)	20.83[ij]	79.16[bc]	0.0[i]	8.33[k]	91.66[ab]	0.0[i]
Interruption by one day at RT (T2)	4.16[ki]	79.16[bc]	16.66[j]	0.0[i]	37.50[fg]	62.50[de]
Interruption by two days at RT (T3)	0.0[i]	70.83[cd]	29.16[gh]	0.0[i]	41.66[f]	58.33[e]
Interruption by three days at RT (T4)	0.0[i]	25.00[hi]	75.00[c]	0.0[i]	20.83[ij]	79.16[bc]
Fruits stored at RT continuously (control) (T5)	0.0[i]	0.0[i]	100[a]	0.0[i]	0.0[i]	100[a]

Mean values followed by similar letters are not significantly different at the % level according to Duncan's multiple range test. *RT (Room temperature

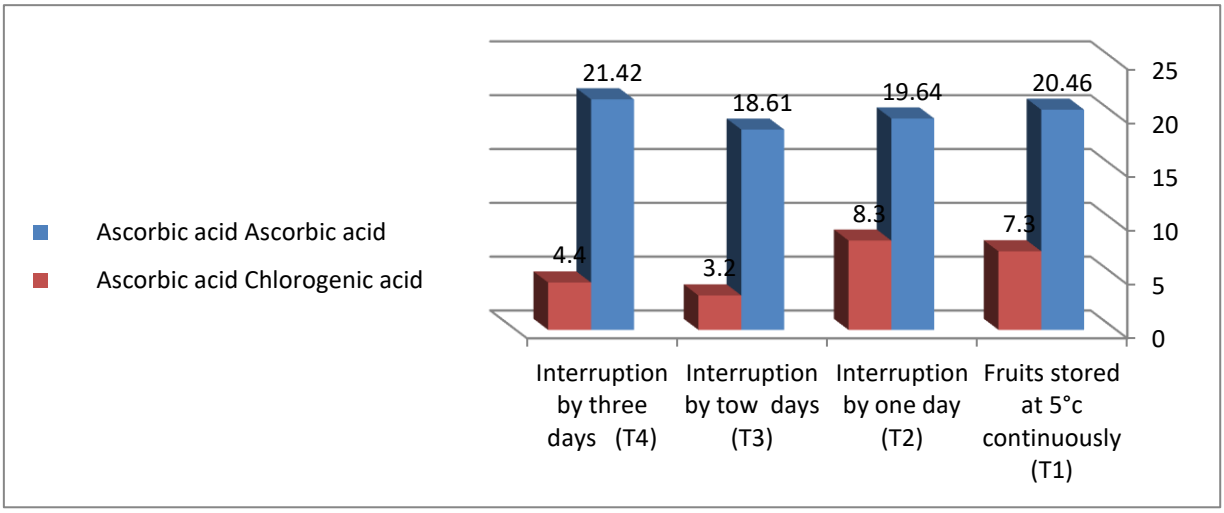

Fig. 2. a: Chloorogenic acid and ascorbic acid content of chilled fruits after storge at low temperature for 21 days
L.S.D. at (0.05) = 2.32
- Treatment (T5) storage at room temperature not included

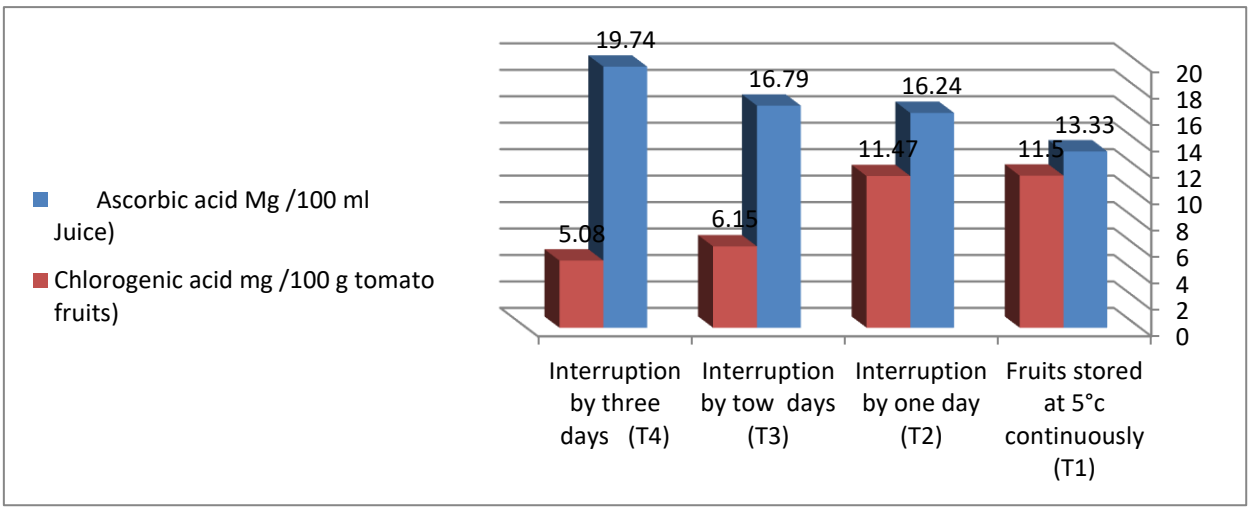

Fig.2. b: Chloorogenic acid and ascorbic acid content of chilled fruits after displaying for 3 days at room temperature
L.S.D. at (0.05) = 2.32
- Treatment (T5) storage at room temperature not included

4. Conclusion

This study evaluated the effectiveness of intermittent warming (IW) temperatures in reducing chilling injury (CI) development and improving the quality of tomato fruits. The results demonstrated that IW significantly minimized CI symptoms. Tomato fruit tolerance to CI improved with an increase in the frequency of warm temperature periods during low-temperature storage, indicating the potential benefits of alternate storage temperatures. Additionally, IW positively impacted fruit quality during low-temperature storage, highlighting its potential as an effective strategy for enhancing tomato fruit preservation.

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