coatings

MDPI

Article

Effect of Harvesting Stages and Calcium Chloride Application on Postharvest Quality of Tomato Fruits

Mohammad Nurun Nabi Mazumder [1,2], Azizah Misran [1,*], Phebe Ding [1], Puteri Edaroyati Megat Wahab [1] and Azhar Mohamad [3]

[1] Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang 43400, Malaysia; mnnmbina@gmail.com (M.N.N.M.); phebe@upm.edu.my (P.D.); putri@upm.edu.my (P.E.M.W.)
[2] Horticulture Division, Bangladesh Institute of Nuclear Agriculture, Bangladesh Agricultural University Campus, Mymensingh 2202, Bangladesh
[3] Malaysian Nuclear Agency, Kajang 43000, Malaysia; azhar_m@nuclearmalaysia.gov.my
* Correspondence: azizahm@upm.edu.my; Tel.: +60-3-97694998

Abstract: Tomatoes are a good source of vitamins, minerals, antioxidants, and enzymes, which are beneficial to human health. They are one of the most commercially high-value vegetable crops that experience a huge postharvest loss after harvest. The present experiment is conducted to investigate the effect of different maturity stages (mature green, breaker, and half-ripe stage), pre- and post-harvest treatment with different concentrations (0.0%, 1.0%, 1.5%, and 2.0%, *w*/*v*) of calcium chloride ($CaCl_2$) on the postharvest performance, antioxidant and enzymatic activity of lowland tomato fruits, stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Tomato fruit of mature green stage treated with 2% $CaCl_2$ significantly (p = 0.05) declined the ethylene production (15.53%), weight loss (16.43%), and delayed color development by slowly synthesizes the lycopene content as well as extended the shelf life. The maximum amount of total phenolic content (TPC) was demonstrated at the highest level of $CaCl_2$ (2%) after 20 days of storage life at ambient conditions. The concentration of $CaCl_2$ influenced the activity of different plant defense enzymes, and the higher doses of $CaCl_2$ (2%) accelerated the activity of peroxidase (POD) (13%), polyphenol oxidase (POP) (7.3%), and phenylalanine ammonia-lyase (PAL) (8.5%) relative to that of the control samples. Therefore, the tomato producers and traders could extend the storage duration of tomato fruits by harvesting at the mature green stage and applying 2% $CaCl_2$ in both pre-and postharvest at ambient storage conditions.

Keywords: tomato; harvesting stages; calcium chloride; quality; antioxidant; enzyme activity; shelf life

Citation: Mazumder, M.N.N.; Misran, A.; Ding, P.; Wahab, P.E.M.; Mohamad, A. Effect of Harvesting Stages and Calcium Chloride Application on Postharvest Quality of Tomato Fruits. *Coatings* **2021**, *11*, 1445. https://doi.org/10.3390/coatings11121445

Academic Editor: Raffaele Porta

Received: 21 September 2021
Accepted: 29 October 2021
Published: 24 November 2021

1. Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the family *Solanaceae* and is the most popular, nutritive, and extensively grown versatile vegetable crop, with production ranking second just after potatoes among the horticultural crops all over the world [1]. Tomato fruits are mostly potential income-generating crops for small and medium-scale farmers, facilitating employment opportunities during production, processing, and within the whole supply chain from farm to fork [2]. Tomato-based functional foods are rich in vitamins (C and A), carotenoids (β-carotene and lycopene), minerals, many phytochemicals, and antioxidant compounds, and these can effectively decline the risk of cardiovascular diseases, cancer and heart disease [3].

Tomato fruits are climacteric in nature, becoming highly perishable during the ripening process. In most cases, the shelf life of tomato fruit is only one and a half weeks after harvest, and it is challenging to maintain the quality during and after harvest [4]. The magnitude of postharvest losses of tomato fruits is high in many developing parts of the

world, as losses occur due to poor agronomic practices, incompetent postharvest handlings methods, inappropriate harvesting stage, improper transportation facility, the unfriendly environment in storage, and infestation of insects [5]. The main obstacle in postharvest shelf life and quality maintenance of tomato fruits is physiological deterioration, consequences of oxidation mechanisms, and the scarcity of convenient postharvest treatments that can decline the deterioration during storage [6].

The essential plant nutrient calcium (Ca) plays a significant role to improve the physical and physiological quality attributes of tomato fruits by increasing the fruit firmness, declining the physiological disorders, delaying the color development process, and extending the shelf life at storage conditions [7]. Numerous physiological and biochemical mechanisms of tomato fruits were believed to be controlled by affiliation of calcium ion (Ca^{2+}) by declining the rate of respiration and ethylene production due to the increasing levels of Ca^{2+} in the fruit matrix [8]. Therefore, the postharvest spraying with $CaCl_2$ showed higher in firmness, lower rate of ethylene and respiration; as a result, fruits showed the delay senescence [9].

As a climacteric fruit, tomato can be harvested at different stages of maturity, for instance, green mature, breaker, turning, pink or light red, and red ripen stage [10]. These maturity stages significantly impact fruits' physiological process, postharvest performance, and sensory attributes after harvest [11]. Tomato fruits are harvested at inappropriate maturity stages, responsible mainly for the postharvest losses in developing countries [10]. The immature fruits are susceptible to physiological injury, shrinkage, and poor quality where, the over-mature fruits are more prone to physical damage, biochemical activity toward senescence, disease, and insect infestation during storage [12,13].

The application of different concentrations (0.0%, 2.0%) of $CaCl_2$ showed that the tomato fruits at breaker stage with $CaCl_2$ application extended the shelf life by producing the lowest amount of ethylene, declining respiration rate, and dropping off the weight loss [14]. Significant reduction of ethylene production and the rate of respiration in tomato fruits treated with $CaCl_2$ or the combination of cactus mucilage extended the shelf life in storage conditions by declining the physiological weight loss [11]. The firmness of the fruit was investigated under Transmission Electron Microscopy (TEM) showed that fruits treated with 1.5% and 2.0% $CaCl_2$ had been maintaining the rigidity of middle lamella after three weeks of storage where it disappeared to the corresponding control samples within the same storage duration [15].

The $CaCl_2$ as well as the calcium lactate also can be used for the postharvest extension of shelf life whereas, the application of pre- and post-harvest $CaCl_2$ is a low cost, eco-friendly, and easy to perform by any level of tomato growers. The use of $CaCl_2$ treatments and proper harvesting stages to extend the shelf life of MT-3 lowland tomato fruits by retaining the antioxidants and plant defense enzymes activity could be a new arena of horticultural research in low land areas. Therefore, the objective of the study was to investigate the effects of harvesting stages, pre- and post-harvest calcium chloride application on postharvest performance, antioxidants, and defense-related enzyme activities of MT-3 lowland tomato fruits.

2. Materials and Methods

2.1. Experimental Site and Duration

This experiment was conducted in the greenhouse (8C) at Agro-Tech Unit, University Agriculture Park in Ladang 15, under the Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia (3°00′21.34″ N; 101°42′15.06″ E, 37 m elevation) from June 2020 to September 2020. Malaysia's lowland areas represent the plant responses to high ambient temperature and high humid conditions. The average air temperature was 33.24 °C (36.45 °C inside the glass house), with an average relative humidity 78.84% (73.57% inside the glasshouse).

2.2. Plant and Planting Materials

The seeds of MT-3 tomato variety were used in the present experiment, the high-yielding tomato variety released from the Malaysia Research and Development Institute (MARDI). The medium of germination was peat moss, and bio-soil (3:1) and Coco peat (2.5 kg/bag) were used as the planting media for tomato plants [16]. According to the standards spacing, tomato seedlings were transplanted at 60 cm between plants and 75 cm between rows.

2.3. Harvesting of Tomato Fruits

Different harvesting stages of tomato fruit—such as the mature green stage (stage 2), breaker stage (stage 3), and half-ripe stage (stage 4) [17] of MT-3 cultivars—were harvested from the experimental plot during August 2020. The tomato fruit were harvested manually and selected for uniformity of size, color, free from diseases, bruises, and blemishes for experimental purposes to investigate the quality, shelf life, and postharvest performance.

2.4. Application of Calcium Chloride ($CaCl_2$)

Harvested fruit (25 fruits in each group) were imposed the postharvest treatments with different percent (w/v) of $CaCl_2$ (A.R grade), (T_0 = 0.0% (dipping in water), T_1 = 1.0% (10 g $CaCl_2$ + 1000 mL water), T_2 = 1.5% (15 g $CaCl_2$ + 1000 mL water) and T_3 = 2.0% (20 g $CaCl_2$ + 1000 mL water) w/v) as direct dip for 10 min along with Tween 20 (0.03% v/v). The tomato fruits were then dried at room temperature (28 ± 2 °C and 75 ± 5% RH) and transferred to the paper carton boxes with a specially designed polybag for postharvest performance observations.

2.5. Postharvest Performance Evaluation

2.5.1. Respiration and Ethylene Production

The harvested tomato fruit (1 fruit in each container) were put in a 1.9 L airtight plastic container purged with airtight. A 1 mL headspace gas sample was removed from the incubation chamber with a gastight syringe and injected into the GC (Gas Chromatograph, Clarus 500, PerkinElmer instrument, Waltham, MA, USA) to assess CO_2 for respiration and ethylene production [18]. The respiration rate was determined by measuring the CO_2 concentration emitted in the outflow from each chamber after 2 h at ambient temperature (25–30 °C) [19].

2.5.2. Firmness

The firmness of the tomato fruit was measured using a Universal Testing Machine (Model 5543 load frame, Instron Corp., Norwood, MA, USA) with a 6 mm diameter cylindrical probe moving at 20 mm/min along with the Instron Merlin software (version M12-13664-EN). The reading for each sample was registered in Newton (N) [20].

2.5.3. Shelf Life

The shelf life was calculated by storing the fruits until the appearance of the final degradation. Total 25 fruits were stored at room temperature (temperature 28 ± 2 °C and 75 ± 5 RH) [21].

2.6. Antioxidant Properties

2.6.1. Ascorbic Acid (Vitamin C)

The dye 2, 6–dichlorophenol-indophenol (0.042 g sodium bicarbonate, $NaHCO_3$ mixed with 150 mL distilled water) was used to determine ascorbic acid and make the volume of 200 mL by adding 50 mg of DCPIP followed by the distilled water [22]. Around 2 g of tomato samples were mixed with 20 mL of metaphosphoric acid 2% (w/v), extracted, and filtered with cotton wool. The resulting material was added with metaphosphoric acid to make a final volume of 40 mL. The aliquot (0.5 mL) was mixed with 3 mL 2% (w/v) HPO_3, then 2 mL dye solution was added, and the absorbance was measured at 518 nm

wavelength immediately using UV-spectrophotometer (Spectrophotometer-1510, +24 V DC/4 A, Multiskan GO, Thermo Fisher Scientific Oy, Vantaa, Finland). The amount of ascorbic acid was calculated as the (mg/g) fresh weight.

2.6.2. Lycopene

The different pigments of tomato fruit were determined following methods described by Nagata and Yamashita [23]. A hand homogenizer was used to mix 1.0 g of tomato sample with 15 mL of acetone: hexane (4:6) solvent. The supernatant was removed from the tube while the aliquot was taken into the quartz cuvette. The UV-spectrophotometer was used to measure the absorbance at 663, 645, 505, and 453 nm. The amount of the pigments was calculated by the following equations:

$$\text{Lycopene (mg/100 mL)} = -0.0458\ A_{663} + 0.204\ A_{645} + 0.372\ A_{505} - 0.0806\ A_{453}$$

A_{663}, A_{645}, A_{505}, and A_{453} are the absorbance at 663 nm, 645 nm, 505 nm, and 453 nm, respectively. Data obtained mg/100 mL was further converted as data mg/100 mL × sample volume = data mg/100 g.

2.6.3. Sample Extraction for Antioxidant Determination

A 3 g tomato sample was ground in a pestle and mortar. The ground sample was poured into a dark-colored tube containing 8 mL of 80% (*v*/*v*) methanol, then placed on an orbital shaker and shaken for one hour at 180 rpm. The mixture was then filtered through No. 1 Whatman filter paper, and the supernatant was ready for DPPH (2,2-diphenyl-1-picrylhydrazyl) and TPC (total phenolic content) determination. The supernatant was stored in the chiller pending the time the experiment would be carried out, and it is a slight modification of the methods reported by [24].

Total Phenolic Content (TPC)

TPC was determined by slightly altering the methods defined by Musa et al. [25]. After mixing 150 µL of supernatant with 750 µL of 10% Folin–Ciocalteu reagent (FCR) and incubating for 5 min, 600 µL of 7.5% (*w*/*v*) sodium carbonate was added (Na_2CO_3). The supernatant of 150 µL was mixed with 750 µL of 10% Folin–Ciocalteu reagent (FCR) and incubated for 5 min. After adding 600 µL of 7.5% (*w*/*v*) sodium carbonate (Na_2CO_3), the mixture was further incubated for another 30 min in the dark. After 30 min of incubation, the absorbance was taken at 765 nm wavelength by UV-spectrophotometer. The results were expressed as mg of Gallic acid equivalents per 100 g of fresh sample (mg GA/100 g of FW).

DPPH Radical Scavenging Assay

The antioxidant activity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging methods described by Musa et al. [25]. The 1 mM DPPH stock solution was made by dissolving 40 mg of DPPH in 100 mL of 80% methanol and kept in a chiller until required. The supernatant of about 1 mL was mixed with 1 mL of 1 mM DPPH and incubated for 30 min in the dark. The absorbance was measured at 517 nm with a UV spectrophotometer immediately after incubation, and the percentage of antioxidant activity was calculated using the formula:

$$\text{DPPH scavenging activity (\%)} = (A_{\text{blank}} - A_{\text{sample}/}A_{\text{blank}}) \times 100$$

where, A is for absorbance.

2.7. Determination of Defence Enzyme Activities

The defense enzyme activity of POD, PPO, and PAL was determined by extracting the tomato fruit tissues following the slightly modified methods defined by Li et al. [26], Soares et al. [27], and Rivero et al. [28] along with the corresponding enzymes assay kit.

2.7.1. Peroxidase (POD) (EC 1.11.1.7) Assay

A peroxidase (POD) assay kit (Producer: Solarbio Biochemical assay Division, Beijing, China) was used for the extraction solution, and the solution I, II, and III were placed in a 96-well micro plate at 25 °C for 10 min before determination [29]. About 0.1 g of tomato fruit tissue was mixed with 1 mL extraction solution (supplied peroxidase assay kit) and completely ground on an ice bar and then centrifuged at 4 °C, 8000 rpm for 10 min. About 5 μL of sample extract along with 60 μL distilled water was mixed with 120, 30, and 30 μL solutions I, II, and III respectively, mixed well immediately, then the time of reaction was recorded. The mixture was measured at 470 nm at 30 s (A1) and 1 min 30 s (A2), then calculated as ΔA = A2 − A1. Unit of peroxidase activity was defined as producing a 0.01 absorbance changed at 470 nm per minute in 1 mL reaction volume for each gram of tissue expressed as:

$$\text{POD (U/g fresh weight)} = \Delta A \times Vrv/(W \times Vsv/Vs)/0.005/T = 9800 \times \Delta A/W$$

where, Vrv: Total reaction volume, 0.245 mL; Vsv: Total supernatant volume, 0.005 mL; Vs: Extraction Solution volume, 1 mL; T: Reaction time, 1 min; W: Sample weight, 0.1 g.

2.7.2. Polyphenol Oxidase (PPO) (E.C1.14.18.1)

Polyphenol oxidase (PPO) assay kit (Producer: Solarbio Biochemical assay Division, Beijing, China) supplied the extract solution, reagents I, and reagent II. About 1 mL extract solution was mixed with 0.1 g fruit tissues and then homogenized on the ice bar. The sample was then centrifuged at 8000 rpm with 4 °C for 10 min and the supernatant was used for spectrophotometric analysis [29]. In the test tube, 50 μL of the sample extract were mixed with 200 μL of reagent I and 50 μL of reagent II, respectively; in the contrast tube (as a control), 50 μL of the boiled sample were added along with the reagents, and the samples were heated in boiled water for 10 min. The mixture was centrifuged at 5000 rpm for 10 min and the absorbance of the test tube (AT) and contrast tube (AC) measured at 410 nm wavelength and then calculated as ΔA = AT − AC. One g tissue per minute changes the absorbance to 0.1 of 410 nm in 1 mL reaction system was defined as an enzyme activity unit expressed as:

$$\text{PPO (U/g)} = \Delta A/0.005 \times VRT/(W/VST \times VS)/T = 120 \times \Delta A/W$$

where, VRT: Reaction total volume, 0.3 mL; VS: Sample volume, 0.05 mL; VST: Extract solution volume, 1 mL; W: Sample weight, 0.1 g; T: Reaction time, 10 min.

2.7.3. Phenylalanine Ammonia-Lyase (PAL) (E.C.4.1.3.5)

Phenylalanine ammonia-lyase (PAL) assay kit (Producer: Solarbio Biochemical assay Division, Beijing, China) supplied the extract solution, reagents 1, reagent 2 and reagent 3. About 1 mL of extract solution was added into 0.1 g of fruit tissue and ground on an ice pack. The sample was then centrifuged at 10,000 rpm, 4 °C for 10 min and the supernatant was used for the enzyme analysis [29]. In test tube, 5 μL of sample extract were mixed with 145 μL of reagent 1 and 40 μL of reagent 2, where 145 μL of reagent 1 and 40 μL of reagent 2 were added without sample in blank tube then mixed in 30 °C hot water bath for 30 min. After boiling, 10 μL of Reagent 3 was added to both samples, mixed thoroughly, and incubate for another 10 min. The absorbance of A1 (test tube) and A2 (blank tube) were determined at 290 nm and calculated as ΔA = A1 − A2. One unit of enzyme activity is defined as 1 g tissue changing 0.05 absorbance at 290 nm per minute in the reaction system expressed as:

$$\text{PAL (U/g)} = \Delta A \times Vsv/Vs/T/0.05/W = 133.4 \times \Delta A/W$$

W: tissue weight, 0.1 g; Vs: sample volume (mL), 5 μL = 0.005 mL; Vsv: extraction volume, 1 mL; T: reaction time (min), 30 min.

2.8. Experimental Design and Statistical Analysis

The experiment was conducted using CRD (Completely Randomized Design) design with a single factor in the glasshouse experiment and CRD design with three factors in the quality analysis as plant performance and CRD design with three factors in the postharvest performance experiment in the laboratory. There was a single variety, MT-3, and a single concentration (2%) of $CaCl_2$ in the glasshouse experiment was performed. The post-harvest experiment were done with 3 harvesting stages namely, green mature stage (stage 2), breaker stage (stage 3) and half-ripe stage (stage 4), postharvest dipping with four concentration of $CaCl_2$, (T_0 = 0.0% (dipping in water), T_1 = 1.0% (10 g $CaCl_2$ with 1000 mL water), T_2 = 1.5% (15 g $CaCl_2$ with 1000 mL water) and T_3 = 2.0% (20 g $CaCl_2$ with 1000 mL water) as w/v) along with 0, 5, 10, 15 and 20 days intervals of data collection which was further replicated with 4 times. All the observations were (4 × 3 × 4) = 48. The data analysis was performed using ANOVA using the SAS software (version 9.4) following CRD design and three factors [30]. The postharvest performance experiment's data of disease incidence, disease severity, and visual symptoms were transformed (arc sine data transformation). The treatment means were compared by protected Least Significant Difference (LSD) at the 5% probability level (p = 0.05). The combined analysis was also done to determine the relationships among the variables of different varieties, different concentrations of calcium chloride, and the different intervals of days for the postharvest performance experiment.

3. Results and Discussions

3.1. Postharvest Performance Evaluation

This study investigated three factors, including harvesting stages, $CaCl_2$ concentrations, and storage durations. The three-way ANOVA tables were generated for each quality parameter analyzed, while the significance and interaction factors were also examined. The significance of all factors was observed statistically according to their p-value (p = 0.05). When there is significant interaction among the factors on the quality parameter, an interaction graph was plotted.

3.1.1. Respiration Rate and Ethylene Production

The three-way interaction among the factors of harvesting stages, concentrations of $CaCl_2$, and different storage durations on the rate of ethylene production was highly significant at p = 0.05 (Table 1).

The results revealed that the ethylene production of MT-3 tomato fruit at the mature green stage was the lowest initially and reached maximum after 5 days of storage duration for control and lower doses of $CaCl_2$-treated samples compared to the higher doses samples (Figure 1). After 5 days, the ethylene production was going down. The higher doses (1.5% and 2%) of $CaCl_2$ produced the lowest ethylene production compared to the lower doses (1% $CaCl_2$) and control samples respectively at 20 days of storage duration.

The ethylene production of tomato fruit at the breaker and half-ripe stages were observed as different than the mature green stage. It was maximum during the initial time of storage and declined with the extension of shelf life up to 20 days (Figure 1). The interaction of harvesting stages, storage durations, and higher doses of $CaCl_2$ (1.5% and 2%) exhibited better results than that in lower doses and control samples, which produced the lowest ethylene at the breaker and half-ripe stage up to 20 days of storage period.

Ethylene is considered the major ripening stimulant which is synthesized during maturity stage in the field or in storage conditions of fruits; as a result, it starts softening of the tomato, leading to insects, disease infestation, and then senescence [31]. The rate of ethylene production in tomato fruit treated with $CaCl_2$ was significantly lower relative to that in untreated fruits. As a result, the fruits took more time to reach the climacteric peak, and the magnitude of the climacteric peak was lowered to a certain extent by $CaCl_2$ treatments [32]. The reasons for the delay of skin color development in calcium-treated tomato fruit may be the effects of $CaCl_2$ on the ethylene-producing cycle, which inhibits the

synthesis of lycopene during ripening [33]. The direct application of $CaCl_2$ anticipated that calcium delayed the rate of ethylene emission by avoiding the solubilization of calcium-binding sites in fruit cell walls which stimulate the ethylene generating process in plasma membrane [34].

Table 1. Main and interaction effects of harvesting stages (Mature green S1, Breaker S2, and Half ripe S3), concentrations of $CaCl_2$ (0, 1%, 1.5% and 2, *w*/*v*), and storage time (5, 10, 15, and 20 days) on ethylene production, respiration rate, and fruit firmness stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH).

Treatments	Ethylene Production (μL C_2H_4/kg/h)	Respiration Rate (mL CO_2/kg/h)	Firmness (N)
Harvesting Stages (HS)	-	-	-
S1	169.22 b	2.21 a	16.66 a
S2	309.15 a	2.30 a	11.51 b
S3	312.70 a	2.22 a	8.37 c
LSD	25.73	0.14	0.92
$CaCl_2$ (*w*/*v*, %)	-	-	-
0	296.80 a	2.23 b	10.75 c
1	294.37 a	2.45 a	11.33 c
1.5	212.95 c	2.05 c	12.67 b
2	250.65 b	2.24 b	13.96 a
LSD	29.71	0.16	1.06
Storage Time (days) (ST)	-	-	-
0	364.42 a	3.03 a	25.54 a
5	329.71 b	3.13 a	15.08 b
10	236.21 c	1.89 b	9.79 c
15	208.03 cd	1.62 c	6.02 d
20	180.07 d	1.54 c	4.47 e
LSD	33.22	0.18	1.18
Interaction	-	-	-
(HS × $CaCl_2$)	*	**	**
(HS × ST)	**	*	**
($CaCl_2$ × ST)	**	**	*
(HS × $CaCl_2$ × ST)	**	**	**

Means within a factor and column followed by the same alphabet are not significantly different at $p = 0.05$ by using the LSD test. * = Significant, ** = highly significant, and ns = non-significant.

Tzoutzoukou and Bouranis [35] reported the similar results in apricot that decreased the ethylene synthesis by the application of $CaCl_2$. The decrease in ethylene level was attributed to Ca binding to calmodulin as a calcium-calmodulin complex, which is capable of altering the biological processes of the fruit. Another study showed that calmodulin is involved in the inhibition of ethylene biosynthesis due to the inactivation capability of the calcium-calmodulin complex in the ethylene biosynthesis pathway of tomato fruits [33]. Ethylene production was normally higher in green mature tomato fruits and the lower in higher doses of $CaCl_2$ initially and it was declined compared to the lower doses and control samples [11].

As shown in Figure 2, the respiration rate of tomato fruit at the green mature stage was the lowest at initial time of storage. It peaked after 5 days for both control and lower doses as compared to the higher doses of $CaCl_2$-treated samples (Figure 2). After 5 days, the respiration rate was declined, and higher doses (1.5 and 2% $CaCl_2$) produced the lowest ethylene compared to the lower doses (1% $CaCl_2$) and control samples, respectively, at 20 days of storage duration. The respiration rate of tomato fruits at the breaker stage and half-ripe stage showed different results relative to that in mature green stages, and it was higher initially, which was declined with the extension of storage time of 20 days (Figure 2). The interaction of harvesting stages, storage times, and the higher doses of $CaCl_2$ (1.5

and 2%) exhibited better results than the lower doses and control samples, whereas the lower respiration rate was observed at the breaker and half-ripe stage up to 20 days of storage life.

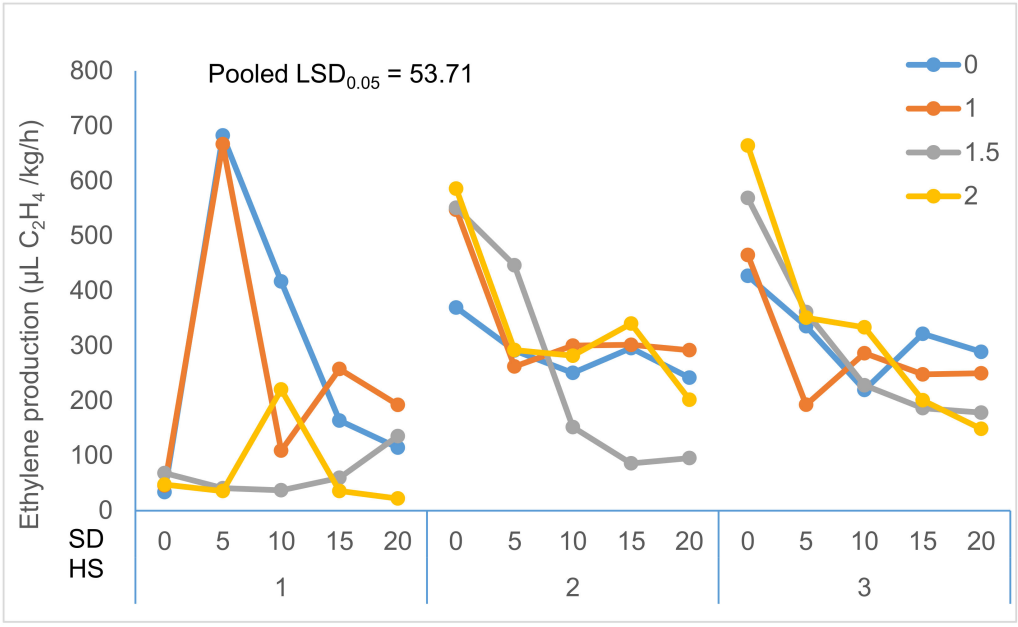

Figure 1. The interaction effects of $CaCl_2$ (0, 1%, 1.5%, and 2%, *w*/*v*), harvesting stages (HS: 1 = green mature, 2 = breaker and 3 = half ripe) and storage durations (SD: 0, 5, 10, 15 and 20 days) on the ethylene production of tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Pooled $LSD_{0.05}$ = 53.71.

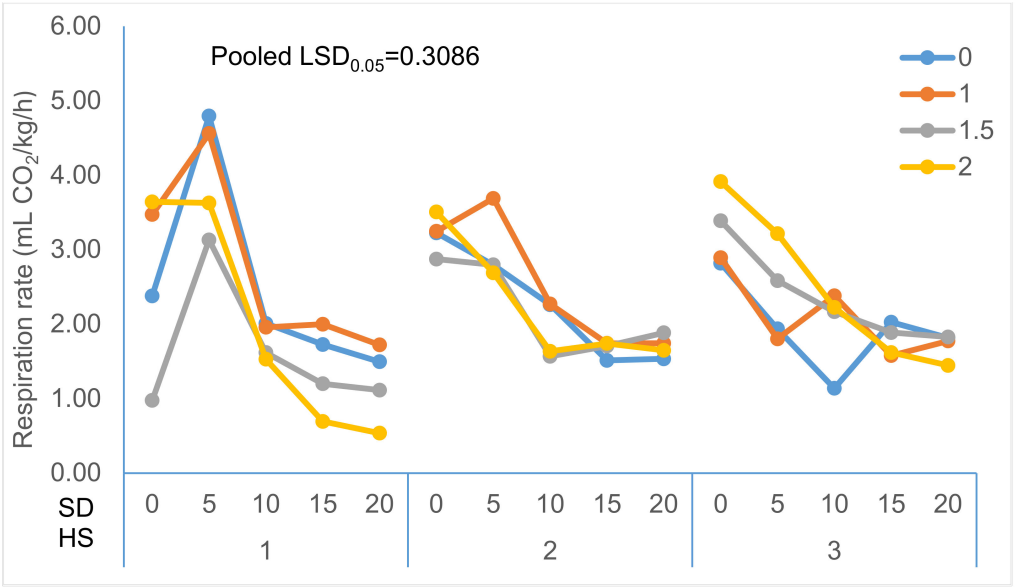

Figure 2. The interaction effects of $CaCl_2$ (0, 1%, 1.5%, and 2%, *w*/*v*), harvesting stages (HS: 1 = green mature, 2 = breaker and 3 = half ripe) and storage durations (SD: 0, 5, 10, 15 and 20 days) on the rate of respiration of tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Pooled $LSD_{0.05}$ = 0.3086.

The process of respiration is a multifaceted consequence where biochemically, it is an oxidative metabolism in plants or fruits matrix and the physiological aspects during respi-

ration; they released the carbon dioxide (CO_2) when they respire [36]. Pre- and postharvest treatments with $CaCl_2$ greatly influenced the respiration rate of fresh fruits and vegetables. The application of 1% $CaCl_2$ demonstrated the lower rate of respiration in tomato fruits [37]. It is also claimed that a sufficient amount of Ca delayed ripening by maintaining the tissue integrity, reducing the rates of transpiration and respiration due to the reduction of the activity softening enzymes [38]. However, the tomato fruits were treated with higher doses of Ca demonstrated lower respiration rates than the corresponding lower doses and control treatments with the advancement of storage duration [39]. Although the rate of respiration is usually higher at the early stages of storage for mature green stages, the higher doses of $CaCl_2$ reduced the rate of respiration compared to lower doses and control samples as storage duration increased [11]. Parallel to this point, reducing respiration rate and ethylene production due to the application of direct fruit or foliar spray of $CaCl_2$ on tomato fruits may decline the disintegration rate of fruit tissues, which maintains the cell wall membrane integrity [40].

3.1.2. Firmness

Data obtained from the present experiment represent that the three-way interaction among the factors of harvesting stages, concentrations of $CaCl_2$, and different storage durations on MT-3 tomato fruit firmness was highly significant at $p = 0.05$ (Table 1).

Tomato fruit firmness was observed as highest at day 0 for the mature green stage, which declined with the increasing of storage time and produced the lowest value at 20 days of storage time. Although the firmness was lower at 0 days for breaker and half-ripe stage than the mature green stage, the declining trends of firmness were similar and reached the lowest level after 20 days of storage duration (Figure 3). Meanwhile, as the shelf life extended, the hardness of the fruit samples declined, and the higher doses of $CaCl_2$ (2.0%) decreased less than the control and other treatment combinations.

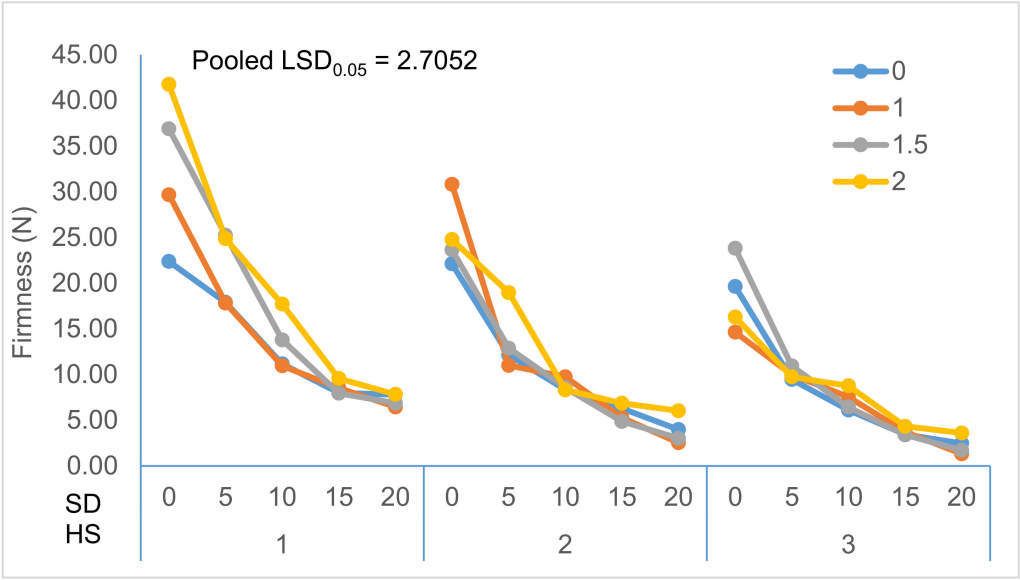

Figure 3. The interaction effects of $CaCl_2$ (0, 1%, 1.5%, and 2%, *w*/*v*), harvesting stages (HS: 1 = green mature, 2 = breaker and 3 = half ripe) and storage durations (SD: 0, 5, 10, 15 and 20 days) on the tomato fruit firmness stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Pooled $LSD_{0.05}$ = 2.7052.

Tomato fruit softening occurred due to the modification of cell polysaccharide components present in the primary cell wall and middle lamella of fruit matrix. This modification leads to loosening and degradation of fruits structure, composition also intracellular materials, which ultimately deteriorated the firmness during the ripening process [4]. Higher

doses of $CaCl_2$ play a vital role in constituting the middle lamellae by binding the polygalacturonic acid, resulting from the rigid firmness, making it more resistant to fruits' physical and microbial damage [14]. According to Tolasa et al. [11], tomato fruits of different maturity stages, treated with the same levels of $CaCl_2$ at the breaker and half ripen stages, resulting in maximum firmness losses at lower dosages of $CaCl_2$. However, the firmness at the breaker and pink stages of tomato fruits treated with higher doses of $CaCl_2$ was observed significantly higher than the lower doses of $CaCl_2$ during the storage time of 12 days [14]. The $CaCl_2$ interacts with pectic acid to form the calcium-pectate complex, resulting from maintaining the cell wall structure by regulating specific enzyme activities enzymes' that cause the softening of fruits [41]. Ca ions also can form salt-bridge with the carboxyl groups of the pectin, making the cell wall not accessible for the enzymes and microbes responsible for the degradation [42].

3.2. Antioxidant Properties

3.2.1. Ascorbic Acid (Vitamin C)

Data recorded from the tomato experiment represents that the three-way interaction among the factors of different harvesting stages, concentrations of $CaCl_2$, and different storage durations on the amount of ascorbic acid were highly significant at $p = 0.05$ (Table 2).

Table 2. Main and interaction effects of harvesting stages (mature green S1, breaker S2, and half ripe S3), concentrations of $CaCl_2$ (0, 1%, 1.5% and 2%, *w*/*v*), and storage durations (0, 5, 10, 15, and 20 days) on ascorbic acid, lycopene, β-carotene, total phenolic content (TPC) and DPPH scavenging activity after harvest, stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH).

Treatment	Ascorbic Acid (mg/g fw)	Lycopene (mg/100 g fw)	TPC (mg/g fw)	DPPH Scav. Activity (%)
Harvesting Stage (HS)	-	-	-	-
S1	0.57 a	2.27 c	0.39 b	36.42 a
S2	0.56 ab	3.01 b	0.40 b	37.44 a
S3	0.54 b	3.65 a	0.45 a	37.60 a
LSD	0.03	0.26	0.04	2.72
$CaCl_2$ (*w*/*v*, %)	-	-	-	-
0	0.56 a	3.05 b	0.39 b	39.56 a
1	0.55 a	3.61 a	0.41 ab	34.22 c
1.5	0.56 a	3.13 b	0.42 ab	38.52 ab
2	0.55 a	2.11 c	0.44 a	36.33 bc
LSD	0.03	0.31	0.05	
Storage Time (days) (ST)	-	-	-	-
0	0.59 a	2.05 c	0.36 b	31.12 d
5	0.58 ab	2.88 b	0.36 b	29.15 d
10	0.57 ab	3.02 b	0.41 b	35.64 c
15	0.55 b	3.03 b	0.47 a	48.28 a
20	0.50 c	3.88 a	0.48 a	41.60 b
LSD	0.03	0.33	0.05	3.52
Interaction	-	-	-	-
(HS × $CaCl_2$)	**	*	*	ns
(HS × ST)	*	**	ns	**
($CaCl_2$ × ST)	**	**	*	**
(HS × $CaCl_2$ × ST)	**	**	ns	Ns

Means within a factor and column followed by the same alphabet are not significantly different at $p = 0.05$ by using the LSD test. * = Significant, ** = highly significant and ns = non-significant.

The amount of ascorbic acid observed in decreasing trend in the most cases of all harvesting stages except the higher doses (2%) of $CaCl_2$ at the breaker stage and the lower doses of $CaCl_2$ (1%) at the half-ripe stage after 10 days of storage duration (Figure 4).

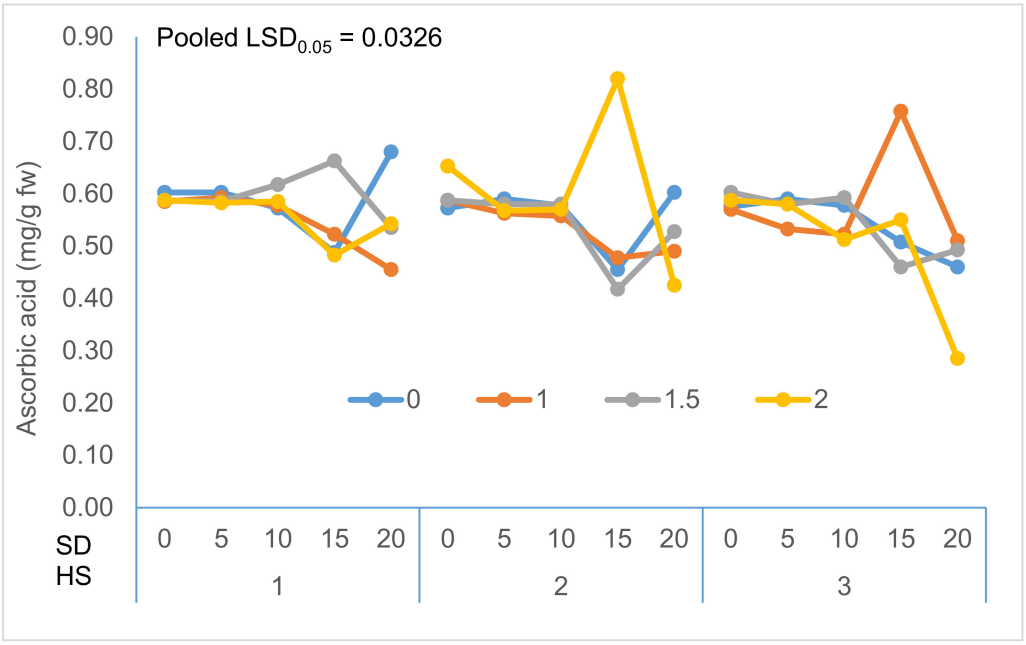

Figure 4. The interaction effects of $CaCl_2$ (0, 1%, 1.5%, and 2%, w/v), harvesting stages (HS: 1 = green mature, 2 = breaker and 3 = half ripe) and storage durations (SD: 0, 5, 10, 15 and 20 days) on ascorbic acid content in tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Pooled $LSD_{0.05}$ = 0.0326.

After 10 days of shelf life, tomato fruits of all stages in most cases of $CaCl_2$ also displayed slightly decreasing trends of ascorbic acid except the higher doses of $CaCl_2$ (2%) at breaker stage and lower doses of $CaCl_2$ (1%) at half ripe stages respectively (Figure 4). Ascorbic acid is the predominant form of vitamin C, an extremely sensitive nutrient, generally used as a sensory and quality indicator [43].

The amount of ascorbic acid in tomato fruit during 30 days of storage was slightly decreased with 3% $CaCl_2$ treatments relative to that in lower doses and control samples [39]. Similar findings were observed by Chepngeno et al. [37] that the ascorbic acid content was 21.63 mg/100 g at the initial storage time and reached the lowest of 16.53 mg/100 g at 9th day of shelf life by the application of 1.5% $CaCl_2$. The postharvest application of $CaCl_2$ declined the ascorbic acid content during the ripening process was investigated by Moneruzzaman et al. [10] in tomatoes, Carvalho and Clemente [44] in broccoli, and Rahman et al. [45] in capsicum. The finding of the experiment was similar to Arthur et al. [46], where tomato fruit were harvested at different maturity stages and treated with varying doses of $CaCl_2$ recorded. The ascorbic acid was 24 mg/100 g at pink, 22 mg/100 g at light red, and significantly lower, 18 mg/100 g at the breaker stage. In agreement with the current study, dipping of tomato fruit in $CaCl_2$ solution remarkably retained the loss of ascorbic acid content during storage which can regulate the oxidative process [47]. However, the decrease in the ascorbic acid content of $CaCl_2$ treated tomato fruits in storage conditions may be attributed to the physiological process such as respiration and transpiration, which leads to the nutrient depletion after harvest [48].

3.2.2. Lycopene

The three-way interaction among the factors of harvesting stages, concentrations of $CaCl_2$, and different storage duration on the lycopene content of tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH) were highly significant at p = 0.05 (Table 2).

The essential pigment of tomato fruits lycopene declined up to 5 days for the green mature stage compared to the other stages where the increasing trends were recorded up to 10 days (Figure 5). It was observed that the lycopene content was further declined after

10 days, which recorded the increasing trends up to 20 days except for the lower doses at the half-ripe stage.

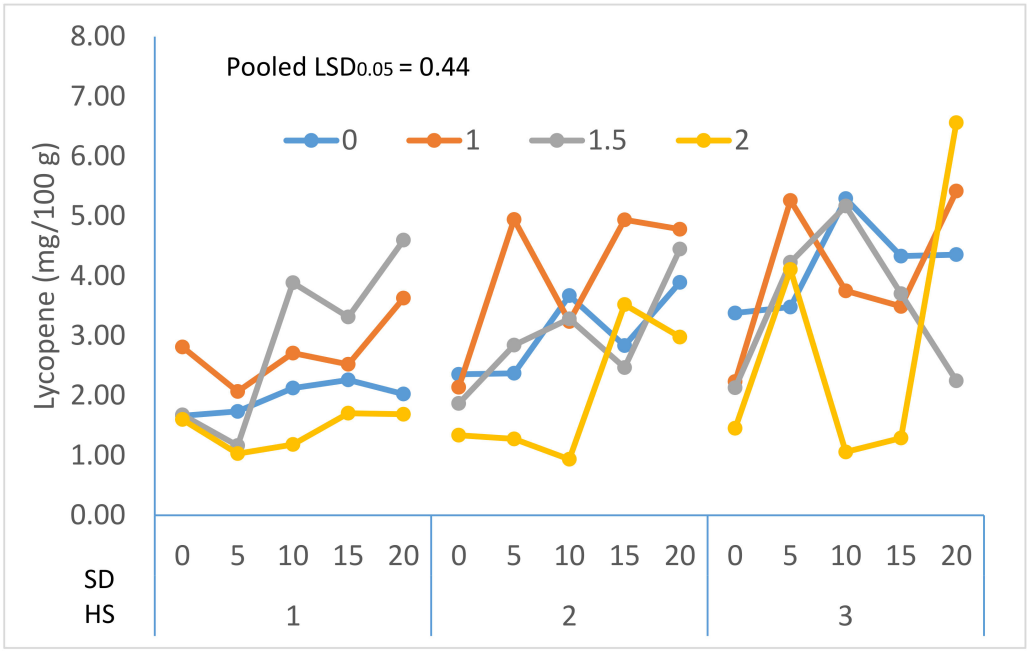

Figure 5. The interaction effects of $CaCl_2$ (0, 1%, 1.5%, and 2%, *w*/*v*), harvesting stages (HS: 1 = green mature, 2 = breaker and 3 = half-ripe) and storage durations (SD: 0, 5, 10, 15 and 20 days) on lycopene content of tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Pooled $LSD_{0.05}$ = 0.0388.

During the ripening process in tomato fruit, the chlorophyll content decreased while carotenoids content was actively synthesized, mainly lycopene [49]. The lycopene content in tomato fruit treated with different doses of $CaCl_2$, stored over 21 days, progressively increased with the advancement of storage time where, the doses of 1% $CaCl_2$, treatment found effective to control the evolution of non-enzymatic antioxidants like lycopene and carotenoids [8]. Similarly, lycopene contents recorded minimum values of 0.03 mg/100 g in tomato fruits treated with 3% of $CaCl_2$ at the mature green stage of 'Hasir Arun' cultivar of tomato [8]. On the other hand, the tomato fruits were harvested at the pink stage, dipping with 6% $CaCl_2$ for 20 min maintained the postharvest quality by declining the lycopene content, weight loss, and microbial decay [14]. Our findings are similar to Tolasa et al. [11], observed that the tomato fruits of 'Roma VF' harvested at the mature green stage treated with cactus mucilage with $CaCl_2$, significantly declined the lycopene content when stored at ambient condition. The present findings also can be explained by the reports of Abebe and Tola [49], that lycopene content on tomato fruits increased sharply with the advancement of storage duration of $CaCl_2$ treated and untreated samples due to the acceleration of the ripening process. On the other hand, the explanations for the inhibition of calcium treated tomato fruit's skin color development might be the effects of $CaCl_2$ on the ethylene generating mechanisms, which affects the synthesis of lycopene in storage conditions due to the ripening process [33,50]. However, during the ripening process, the changes of lycopene content in fruits and vegetables may be attributed to its reactions with free radicles or oxidizing agents, resulting from the interruption of poly chain, conversion of chloroplast to chromoplasts, and accumulation of lycopene in fruit internal cell wall membrane [51].

3.2.3. Total Phenolic Content (TPC) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

Data in Table 2 represents the three-way interaction among the factors of harvesting stages, concentrations of $CaCl_2$, and different storage duration on the amount of antioxi-

dant; TPC and the antioxidant activity; DPPH were non-significant at $p = 0.05$. Whereas, significant two-way relationships were observed between the harvesting stages × levels of $CaCl_2$ and different levels of $CaCl_2$ × storage duration on TPC.

The amount of TPC was lowest for the mature green and breaker stages, where it was highest at higher doses of $CaCl_2$ (1.5% and 2%) except in the half ripen stage. TPC has recorded the maximum levels for the half-ripe stage with the application of 1.5% $CaCl_2$ compared to the control and other treatments combination (Figure 6).

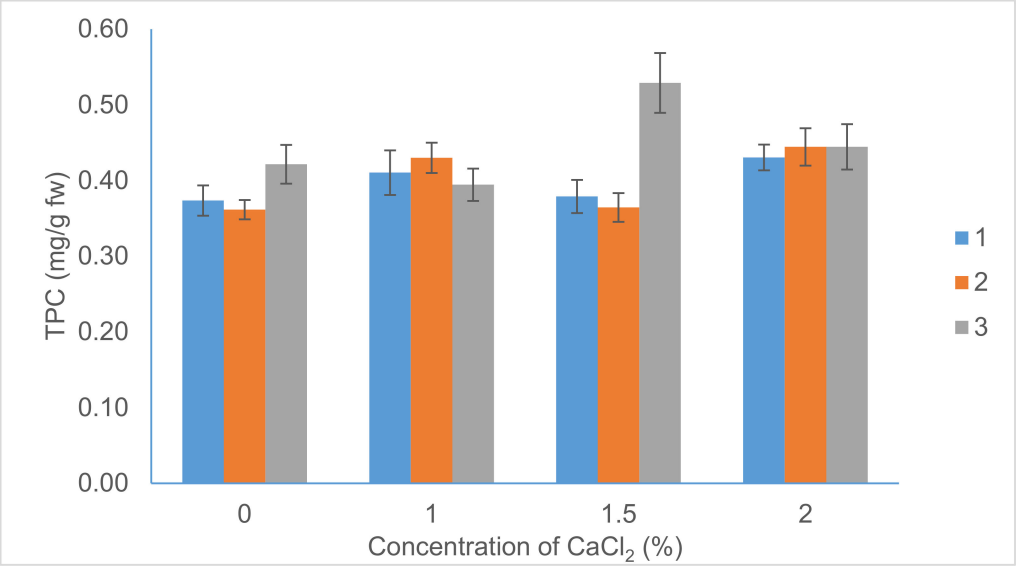

Figure 6. The interaction effects of harvesting stages (1 = green mature, 2 = breaker, and 3 = half-ripe) and $CaCl_2$ (0, 1%, 1.5% and 2%) on TPC of the tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Vertical bars indicate the standard error of means for 4 replicates.

The treatment combination of $CaCl_2$ and storage durations on TPC recorded the lowest for all treatments at the initial storage time, which gradually increased up to 20 days of shelf life except for the doses of 1.5% $CaCl_2$ in tomato fruit. The maximum amount of TPC was illustrated at the highest level of $CaCl_2$ (2%) after 20 days of storage duration, where the lowest was recorded for control and 1.5% $CaCl_2$ treatments, respectively (Figure 7).

The formation of brown substances in fruits and vegetables is usually accompanied by TPC, and the high phenolic content in $CaCl_2$ treated tomato fruits could be attributable to the discharge of phenolic compounds from the treated fruit matrix [52]. Similarly, tomato fruits of 'Hisar Arun (Local)' and 'Kashi Vishesh (Hybrid)' treated with $CaCl_2$ found an increasing trend of TPC and the increasing percentage of $CaCl_2$ from 1% to 5%, also increases the rate of TPC with the advancement of storage duration [8]. The present investigation conforms with Ranjbar et al. [41] reports that the nano-calcium treatment with the doses of 2.5% showed the maximum TPC compared to the control in apple and persimmon fruit [53]. Our findings are in line with those of Addai et al. [24], who found that the amount of TPC had a significant effect on different harvesting stages of papaya fruits, and that the amount of TPC increased as maturity stages progressed. However, $CaCl_2$ increased the polysaccharide content in the fruit cell wall and maintained the strength of the cell membrane, which ultimately prevents the senescence-induced stress and delays the release or oxidize the phenolic compound from the fruit matrix [9].

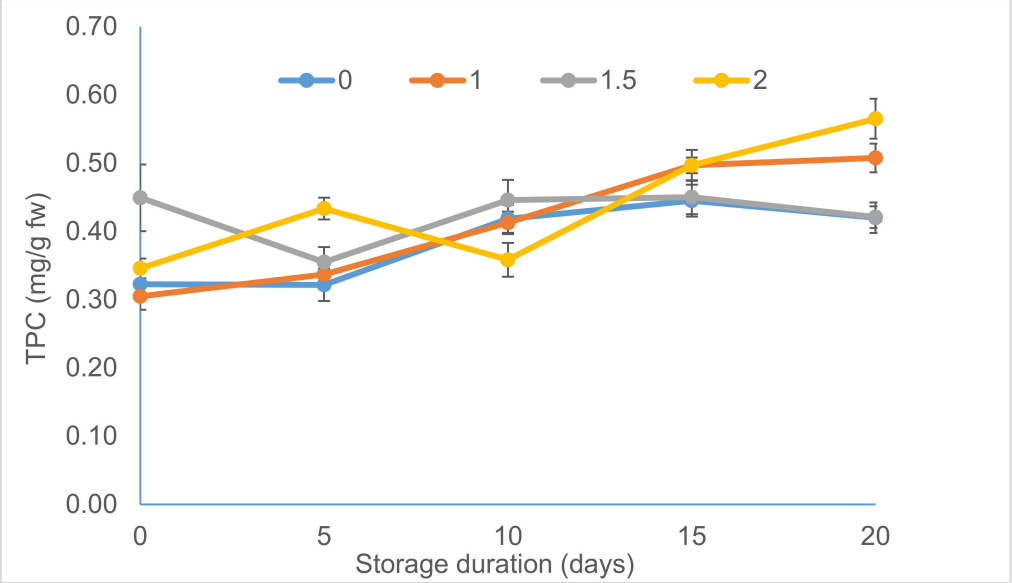

Figure 7. The interaction effects of $CaCl_2$ (0, 1%, 1.5%, and 2%) and storage durations (0, 5, 10, 15, and 20 days) on the amount of TPC of tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Vertical bars indicate the standard error of means for 4 replicates.

Although the three-way interaction was non-significant, significant two-way relationships appeared between the harvesting stages × storage times and different levels of $CaCl_2$ × storage durations on DPPH. The antioxidant activity was lowest for all harvesting stages at the initial storage time, which was increased up to 15 days of storage duration and started to decline afterward except the samples treated with 1.5% $CaCl_2$ at half-ripe stage. The tomato fruit at the half-ripe stage showed different results after 15 days, which was continued the increasing trends compared to other stages up to 20 days of shelf life (Figure 8).

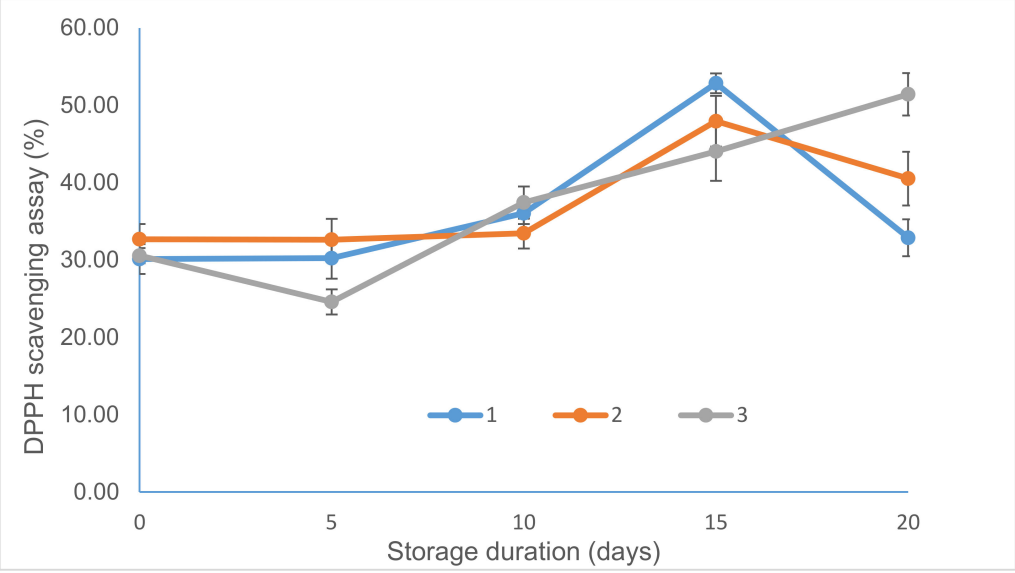

Figure 8. The interaction effects of harvesting stages (1 = green mature, 2 = breaker and 3 = half-ripe) and storage durations (0, 5, 10, 15, and 20 days) on DPPH scavenging activity (%) of tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5%RH). Vertical bars indicate the standard error of means for 4 replicates.

Tomato fruits with 1.5% $CaCl_2$ stated the different results after 15 days, which was continued the increasing trends and delineated the highest DPPH activity relative to the other treatments up to 20 days of shelf life (Figure 9).

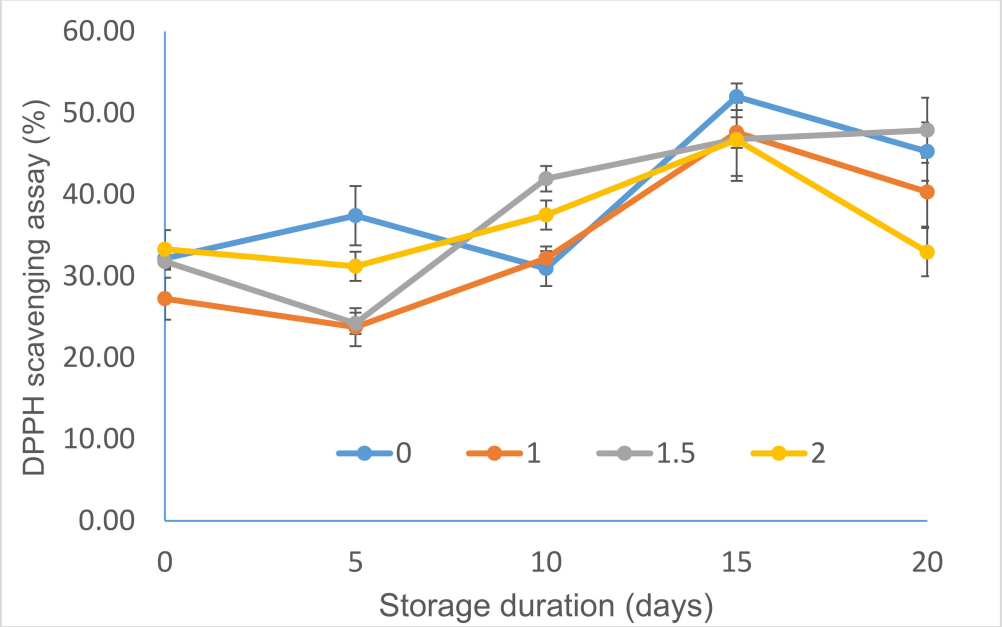

Figure 9. The interaction effects of $CaCl_2$ (0, 1, 1.5, and 2%) and storage durations (0, 5, 10, 15, and 20 days) on DPPH scavenging activity (%) of tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Vertical bars indicate the standard error of means for 4 replicates.

At ambient temperatures, the levels of DPPH on tomato fruit were increasing, which could be attributable to the advanced phases of ripening when the maximal concentration of phenolic compounds occurred in the fruit matrix [54]. The present investigation was similar with the reports of Mishra and Prakash [8], that the tomato fruits of 'Hisar Arun (Local)' and 'Kashi Vishesh (Hybrid)' cultivars treated with $CaCl_2$ found an increasing trend of DPPH up to 14 days of storage time. A similar trend of DPPH was also investigated by Mujtaba et al. [52], who found that the antioxidant activity was higher during the early stages of shelf life for all harvesting stages. However, it decreased at the 30th day of storage when the lycopene and beta-carotene content was lower, consequently decreasing the total antioxidant activity. Parallel to this point, another investigation also recorded by Cano et al. [54], that the lower scavenging activity was illustrated at pink stage of vine tomato. The present findings were similar to the investigation of Addai et al. [24] that the amount of DPPH has significant effects on harvesting stages, which was increased with the advancement of maturity stages.

3.3. Defence Enzymes Activities

The three-way interaction of maturity stages, $CaCl_2$, and storage times on POD activity presented in the Table 3 was highly significant at $p = 0.05$, where it was non-significant for the activity of PPO and PAL stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH).

Table 3. Main and interaction effects of harvesting stages (mature green S1, breaker S2, and half ripe S3), $CaCl_2$ (0, 1, 1.5 and 2%, *w*/*v*), and storage durations (0, 10, and 20 days) on plant defense enzymes (POD, POP, and PAL; unit activity) present in MT-3 tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH).

Factor/Treatment	POD (U/g)	PPO (U/g)	PAL (U/g)
Harvesting Stages (HS)	-	-	-
S1	1546.80 b	86.75 a	68.69 b
S2	1002.00 c	87.32 a	75.21 ab
S3	1958.60 a	85.68 a	81.76 a
LSD	220.34	4.60	10.96
$CaCl_2$ (*w*/*v*, %)	-	-	-
0	1546.10 a	82.63 b	75.95 ab
1	1763.00 a	86.61 ab	76.60 ab
1.5	947.30 b	88.57 a	65.88 b
2	1753.50 a	88.52 a	82.45 a
LSD	254.43	5.32	12.65
Storage Time (day) (ST)	-	-	-
0	1552.70 ab	106.69 a	81.88 a
10	1594.30 a	86.62 b	74.52 ab
20	1360.50 b	66.43 c	69.26 b
LSD	220.34	4.60	10.96
Interaction	-	-	-
HS × $CaCl_2$	*	**	ns
HS × ST	**	Ns	ns
$CaCl_2$ × ST	**	Ns	ns
HS × $CaCl_2$ × ST	**	Ns	ns

Means within a factor and column followed by the same alphabet are not significantly different at $p = 0.05$ by using the LSD test. * = Significant, ** = highly significant and ns = non-significant.

The unit activity of POD performed a similar trend for the green mature and breaker stage where it was minimum at the initial time of storage and increased up to 10 days of storage duration. After 10 days, POD activity was started to decline and continue up to 20 days of storage duration (Figure 10). POD activity for the half-ripe stage investigated different results relative to the other, started declining initially and increasing trend until 20 days of storage duration. The highest activity of POD was illustrated at higher doses of 2% $CaCl_2$ for the half-ripe stage, and the lowest was recorded for the control treatment for the mature green stage after 20 days of shelf life.

The three-way interaction effects on plant defense enzyme activity of PPO were non-significant, where the two-way interaction between harvesting stages and the doses of $CaCl_2$ was highly significant (Table 3). The PPO activity of different harvesting stages of tomato fruit responds differently, where the mature green stage investigated the highest activity at the doses of 1.5% $CaCl_2$ and the lowest at higher doses of 2% $CaCl_2$, respectively. The harvesting stages of breaker and half-ripe illustrated the similar trend of PPO activity where they recorded the lowest at control treatment and the highest at maximum doses of 2% $CaCl_2$ respectively when the tomato fruit were stored at ambient condition (28 ± 2 °C and 75 ± 5% RH) (Figure 11).

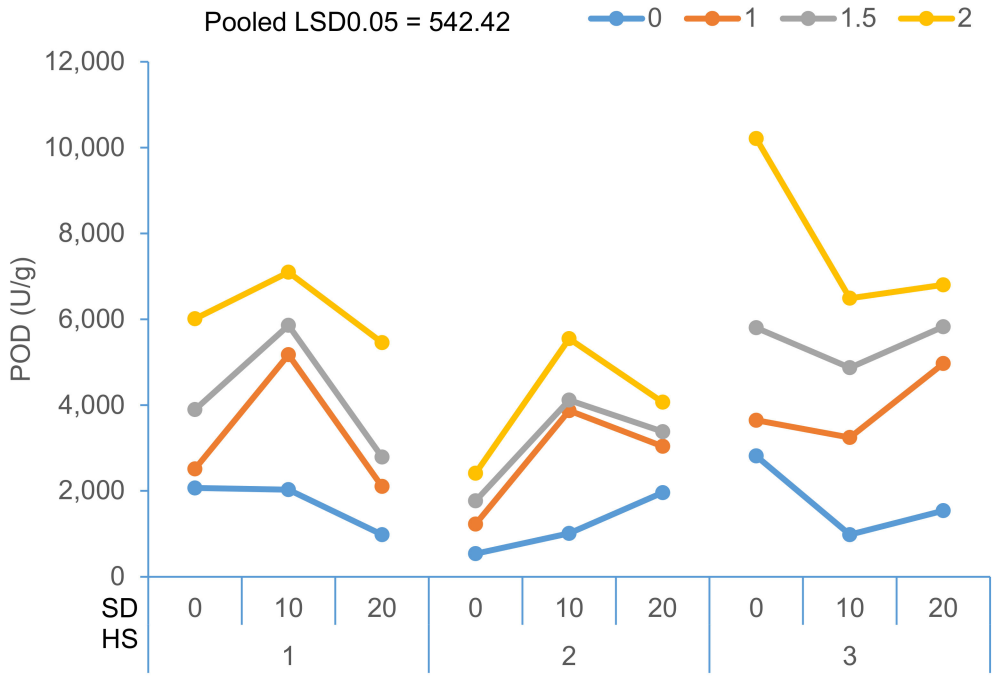

Figure 10. The interaction effects of $CaCl_2$ (0, 1, 1.5, and 2%, *w*/*v*), harvesting stages (HS: 1 = green mature, 2 = breaker and 3 = half-ripe), and storage durations (SD: 0, 10 and 20 days) on POD enzyme activity (unit) of MT-3 tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Pooled $LSD_{0.05}$ = 542.42.

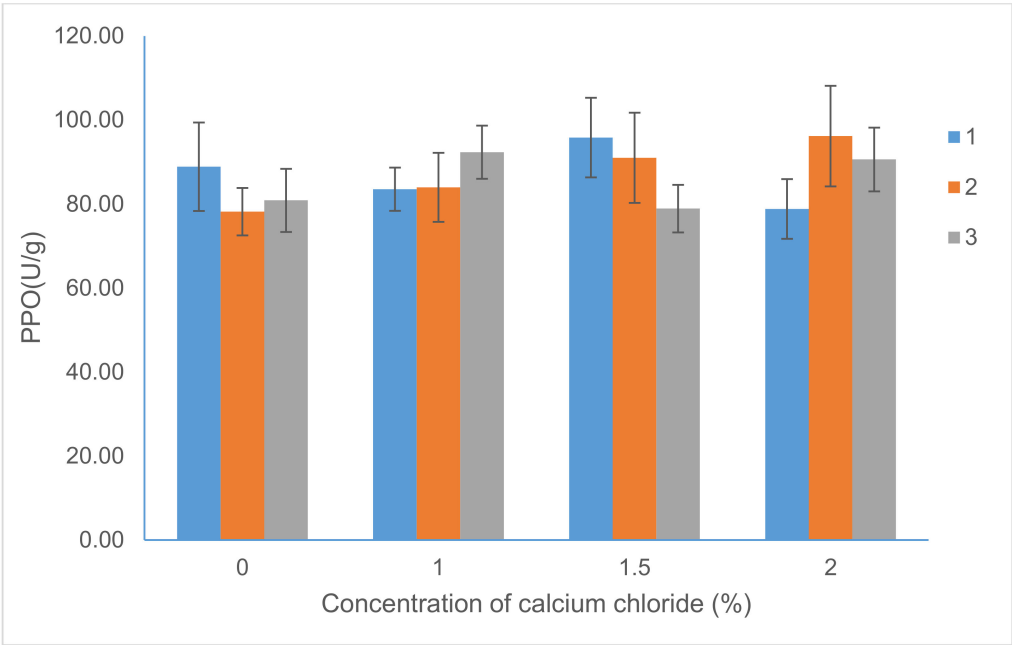

Figure 11. The interaction effects of harvesting stages (1 = green mature, 2 = breaker and 3 = half-ripe) and $CaCl_2$ (0, 1, 1.5, and 2%, *w*/*v*) on PPO enzyme unit activity of MT-3 tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Vertical bars indicate the standard error of means for 4 replicates.

The unit activity of important plant defense enzyme, phenylalanine ammonia-lyase (PAL) was non-significant among the interaction of the treatments. The harvesting stages of

half ripen delineated 8.71% and 19.03% higher PAL activity than the corresponding green mature and breaker stages, respectively (Table 3). The different concentrations of $CaCl_2$ exhibited differently on the activity of PAL where, the higher doses of 2% $CaCl_2$ resulted in the maximum PAL activity of 7.88% higher relative to the control samples (Table 3). Apart from these, plant defense enzymes activity was investigated inversely proportionate with the storage duration. In the case of PAL activity, it was 9.88% and 18.22% lower after 10 days and 20 days of storage duration, respectively, compared to the initial activities of tomato fruits stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). (Table 3).

The essential plant nutrient Ca plays a vital role in plants growth and development system also act as the universal messenger (signaling process), which is involved in several aspects of biotic and abiotic stress in plant or fruit matrix [55]. Some of the earlier investigations revealed that the calcium ion (Ca^{2+}), nitric oxide (NO), and calmodulin (CaM) is responsible for the signaling process required for the ups and down the instruction of the expression of plant defense-related molecules such as phenol, antioxidant and enzymes [56]. In agreement with the current investigation, plant and plant product are treated with different biotic and abiotic elicitor molecules such as $CaCl_{2,}$ significantly encourage the plant's characteristic immune system to overexpress the defense-related enzymes which increased the phenolics and the signaling molecules [57,58]. The present observation conforms to the reports that the direct application of $CaCl_2$ in tomato fruits leads to an increase in the Ca content and protects the membrane lipid degradation, which is responsible for the peroxidation of polyunsaturated fatty acids [59]. The previous study also showed that the application of 0.5% $CaCl_2$ in tomato fruits recorded the extended the number of defense-related enzymes of POD, PPO, PAL, β-1,3-glucanase, and chitinase, coincide with the increased production of antioxidative enzymes like CAT and APX relative to that in untreated control treatments [60]. Similarly, a notable increment of defense enzymes of POD, PPO, PAL, and β-1,3-glucanase was observed by Chandra et al. [61] with the application of $CaCl_2$ in tomatoes. POD and PPO were considered to be involved in strengthening the cell wall by the accumulation of lignin, which leads to protection against various invading pathogens [61]. PAL acts as the entry point enzymes in the phenylpropanoid biosynthesis pathway plays an essential role in phenolic compound synthesis [62], and β-1,3-glucanase is well known for its antifungal activities by hydrolyzing the fungal cell wall [61,63]. It is also claimed that $CaCl_2$ acts as an abiotic elicitor, which declined the postharvest deterioration of fruits by the increasing levels of defense enzymes in pear during storage conditions [63]. It was also found to control the blister blight disease incidence of tea by the accumulation of defense enzymes, phenols, and NO (nitric oxide) [58] and increase the PAL activity leading to the accumulation of phenolics in citrus [64,65].

3.4. Shelf Life (Days)

The present experiment illustrated the interaction data of harvesting stage and $CaCl_2$ were non-significant ($p = 0.05$) on the shelf life of tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Still, they were significantly different among the treatments of varying harvesting stages and different levels of $CaCl_2$ (Table 4).

For the shelf life of the MT-3 tomato fruit, it was recorded that the mature green stage resulted in the highest storage duration, which was 4.73% and 11.81% higher than the breaker and half-ripe stage, respectively (Figure 12). Data showed that the shelf life of tomato fruits was proportionate with the doses of $CaCl_{2,}$ and it was 3.90%, 5.92%, and 9.12% higher at 1%, 1.5%, and 2% concentration of $CaCl_{2,}$ respectively, relative to that in control samples.

Table 4. Main interaction effects of harvesting stages (mature green S1, breaker S2, and half ripe S3) and $CaCl_2$ (0, 1, 1.5, 2%, *w*/*v*) on the shelf life of tomato fruit after harvest stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH).

Factors/Treatments	Shelf Life (Day)
Harvesting Stage (HS)	
S1	24.81 a
S2	23.69 b
S3	22.19 c
LSD	1.03
Calcium (II) chloride, C (%)	
0	22.42 c
1	23.33 bc
1.5	23.83 ab
2	24.67 a
LSD	1.18
Interaction (V × C)	ns

Means followed by the same letter within the column are not significantly different (p = 0.05) at LSD where ns = non-Significant.

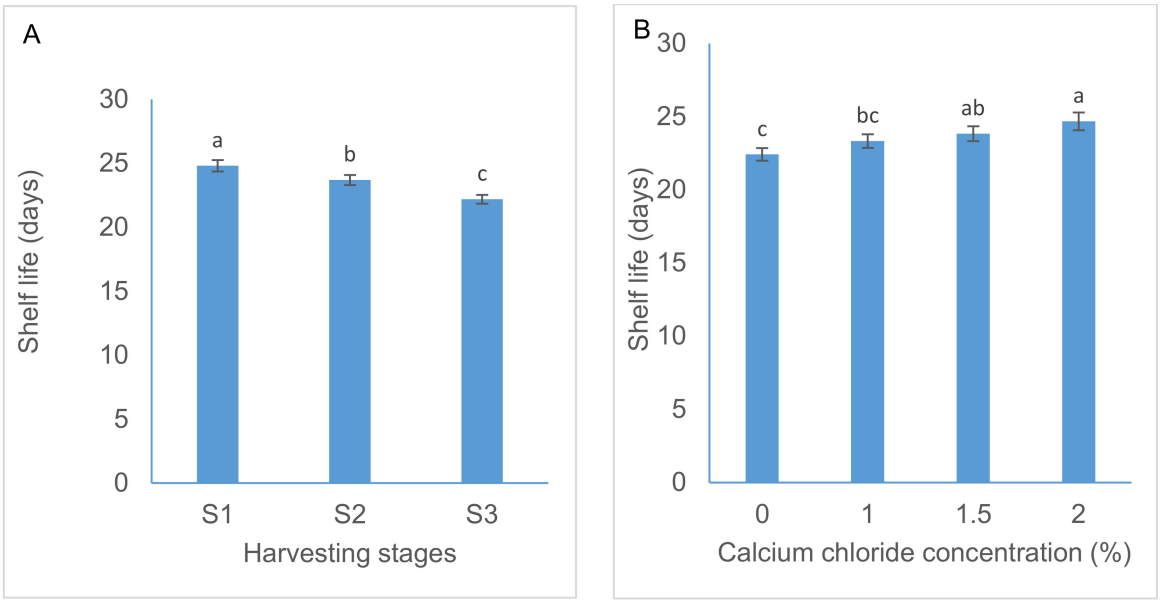

Figure 12. Effects of harvesting stages (**A**) and percent $CaCl_2$ (**B**) on shelf life (days) of tomato fruit stored at ambient temperature (28 ± 2 °C and 75 ± 5% RH). Means followed the same letters are not significant at LSD at p = 0.05, where the vertical bars indicate the standard error of means for four replicates.

Shelf life of MT-3 tomato fruit is influenced by various pre- and post-harvest factors and calculating the number of days required to attain the full ripening stage when the fruit still maintains the acceptable marketing and eating qualities [66]. Marketing and eating quality were visually observed by the different physiological and physicochemical parameters. Tomato fruits, being climacteric in nature, have a short life span after harvesting due to several factors of physical injury during harvest, postharvest disease infection, accelerated ripening, and senescence, which triggered the losses in quality and quantity [4]. Studies showed that tomato fruit treated with 1.0% $CaCl_2$ were found to decline the ethylene production and the rate of respiration; thereby it takes more time to unmask the yellow and red carotenoids resulting in the longer shelf life of treated fruits compared to the control samples [37]. In a similar study, the tomato fruits of 'Roma VF' cultivar harvested at different stages coated with $CaCl_2$ and cactus mucilage was significantly prolonged the

shelf life up to more than two weeks by maintaining the acceptable qualities at the mature green stage relative to that in pink and light red stages including the control treatments respectively [36]. Parallel to this point, a study was carried by Sohail et al. [67] in peach fruit, dipping 5 min with different concentrations of $CaCl_2$, resulting in 3% $CaCl_2$ increasing the storage duration by maintaining the storage the maximum firmness, reducing the decay symptoms, and physiological weight loss.

It is also claimed that tomato fruits harvested at the early mature stage (breaker stage) dipping with 6% $CaCl_2$ for 30 min recorded a significantly longer shelf life compared to the pink stage and lower doses of $CaCl_2$ [46]. The present findings agree with the reports of Madani et al. [15] in papaya, Gharezi et al. [68] in cherry tomato that the lower disease severity in calcium-treated fruits due to delayed ripening, maintaining cell wall integrity, that was confirmed by microscopic observation of middle lamella which was more intact in calcium treated tomato fruits.

4. Conclusions

The postharvest shelf of tomato fruit is principally influenced by the harvesting stages and methods, different types of pre- and post-harvest treatments, as well as storage conditions. The highest concentration of 2% $CaCl_2$ in the mature green stage demonstrated the lowest rate of respiration and ethylene production during the evaluation period of postharvest performance, which facilitates the decline in weight loss by increasing the fruit firmness resulting in the extension of shelf life up to 24 days. Additionally, tomato fruit of three maturity stages showed different results in quality parameters and shelf life from the initial to the final stages of maturation. The half-ripe stage showed more susceptibility to percent weight loss and decreased shelf life. On the other hand, the defense enzymes activity of POD, PPO, and PAL revealed better results in the mature green stage of tomato fruits treated with the higher doses of 2% $CaCl_2$ and retain the activity up to the 24th day by delaying the color development and softening. However, further research is required to explore the potentiality of $CaCl_2$ as a postharvest means of extending shelf life and maintaining the desired quality of different tomato cultivars at different environmental conditions.

Author Contributions: Conceptualization, A.M. (Azizah Misran) and M.N.N.M.; Data Curation, M.N.N.M.; Formal Analysis, A.M. (Azizah Misran) and M.N.N.M.; Funding Acquisition, A.M. (Azizah Misran) and M.N.N.M.; Investigation, A.M. (Azizah Misran), P.D., P.E.M.W. and A.M. (Azhar Mohamad); Methodology, A.M. (Azizah Misran) and M.N.N.M.; Supervision, A.M. (Azizah Misran), P.D., P.E.M.W. and A.M. (Azhar Mohamad).; Validation, A.M. (Azizah Misran), P.D., P.E.M.W. and A.M. (Azhar Mohamad); Visualization, A.M. (Azizah Misran), P.D., P.E.M.W. and A.M. (Azhar Mohamad); Writing—Original Draft, M.N.N.M. and A.M. (Azizah Misran); Writing—Review and Editing, M.N.N.M. and A.M. (Azizah Misran). All authors have read and agreed to the published version of the manuscript.

Funding: This research work was supported by the Bangladesh Agricultural Research Council (BARC) through the research grant of the National Agricultural Technology Programme-Phase II (NATP-2), Bangladesh.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Acknowledgments: We would like to thanks to the Bangladesh Institute of Nuclear Agriculture (BINA) and Universiti Putra Malaysia (UPM) for the continuous support to perform the research activities in Malaysia.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ibrahim, F.A.; Shukla, P.K.; Ramteke, P.W.; John, S.A. Role of calcium chloride on biochemical properties of tomato (*Lycopersicon esculentum*) against different levels of NaCl. *J. Pharmacogn. Phytochem.* **2019**, *8*, 1661–1664.
2. Teka, T.A. Analysis of the effect of maturity stage on the postharvest biochemical quality characteristics of tomato (*Lycopersicon esculentum* Mill.) fruit. *Int. Res. J. Pharm. Appl. Sci.* **2013**, *3*, 180–186.
3. Wabali, V.C.; Esiri, A.; Zitte, L. A sensory assessment of color and textural quality of refrigerated tomatoes preserved with different concentrations of potassium permanganate. *Food Sci. Nutr.* **2017**, *5*, 434–438. [CrossRef]
4. Safari, Z.S.; Ding, P.; Juju Nakasha, J.; Yusoff, S.F. Combining Chitosan and Vanillin to Retain Postharvest Quality of Tomato Fruit during Ambient Temperature Storage. *Coatings* **2020**, *10*, 1222. [CrossRef]
5. Emana, B.; Afari-Sefa, V.; Nenguwo, N.; Ayana, A.; Kebede, D.; Mohammed, H. Characterization of pre-and postharvest losses of the tomato supply chain in Ethiopia. *Agric. Food Secur.* **2017**, *6*, 1–11. [CrossRef]
6. Eça, K.S.; Sartori, T.; Menegalli, F.C. Filmes e coberturas comestíveis contendo antioxidantes-uma revisão. *Braz. J. Food Technol.* **2014**, *17*, 98–112. [CrossRef]
7. Abbasi, N.A.; Zafar, L.; Khan, H.A.; Qureshi, A.A. Effects of naphthalene acetic acid and calcium chloride application on nutrient uptake, growth, yield, and postharvest performance of tomato fruit. *Pak. J. Bot.* **2013**, *45*, 1581–1587.
8. Mishra, S.; Prakash, V. Biochemical changes in calcium chloride treated Hisar Arun (Local) and Kashi Vishesh (Hybrid) cultivars of Tomato fruit. *Curr. Agric. Res. J.* **2018**, *6*, 395. [CrossRef]
9. Shiri, M.A.; Ghasemnezhad, M.; Fatahi Moghadam, J.; Ebrahimi, R. Enhancing and Maintaining Nutritional Quality and Bioactive Compounds of "Hayward" Kiwifruit: Comparison of the Effectiveness of Different $CaCl_2$ Spraying Times. *J. Food Process. Preserv.* **2016**, *40*, 850–862. [CrossRef]
10. Moneruzzaman, K.M.; Hossain, A.B.M.S.; Sani, W.; Saifuddin, M.; Alenazi, M. Effect of harvesting and storage conditions on the postharvest quality of tomato (*Lycopersicon esculentum* Mill) cv. Roma VF. *Aust. J. Crop. Sci.* **2009**, *3*, 113.
11. Tolasa, M.; Gedamu, F.; Woldetsadik, K. Impacts of harvesting stages and pre-storage treatments on shelf life and quality of tomato (*Solanum lycopersicum* L.). *Cogent Food Agric.* **2021**, *7*, 1863620. [CrossRef]
12. Hamdu, I.H.; Yunus, A.; Hardi, I.M. Maturity and Ripening on the Biochemical Characteristics of Three Local Varieties of Tomato. *ADRRI J. Agric. Food Sci.* **2016**, *2*, 1–13.
13. Arah, I.K.; Ahorbo, G.K.; Anku, E.K.; Kumah, E.K.; Amaglo, H. Postharvest handling practices and treatment methods for tomato handlers in developing countries: A mini review. *Adv. Agric.* **2016**, *2016*, 6436945. [CrossRef]
14. Eric, A.; Oduro, I.; Kumah, P. Postharvest quality response of tomato (*Lycopersicon esculentum*, Mill) fruits to different concentrations of calcium chloride at different dip-times. *Am. J. Food Nutr.* **2015**, *5*, 1–8.
15. Madani, B.; Mohamed, M.T.M.; Watkins, C.B.; Kadir, J.; Awang, Y.; Shojaei, T.R. Preharvest calcium chloride sprays affect the ripening of Eksotika II' papaya fruits during cold storage. *Sci. Hortic.* **2014**, *171*, 6–13. [CrossRef]
16. Awang, Y.; Shaharom, A.S.; Mohamad, R.B.; Selamat, A. Chemical and physical characteristics of cocopeat-based media mixtures and their effects on the growth and development of *Celosia cristata*. *Am. J. Agric. Biol. Sci.* **2009**, *4*, 63–71. [CrossRef]
17. Beckles, D.M. Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum* L.) Fruit. *Postharvest Biol. Technol.* **2012**, *63*, 129–140. [CrossRef]
18. Zhu, S.H.; Zhou, J. Effect of nitric oxide on ethylene production in strawberry fruit during storage. *Food Chem.* **2007**, *100*, 1517–1522. [CrossRef]
19. Colombié, S.; Beauvoit, B.; Nazaret, C.; Bénard, C.; Vercambre, G.; Le Gall, S.; Gibon, Y. Respiration climacteric in tomato fruits elucidated by constraint-based modelling. *New Phytol.* **2017**, *213*, 1726–1739. [CrossRef]
20. Batu, A. Determination of acceptable firmness and colour values of tomatoes. *J. Food Eng.* **2004**, *61*, 471–475. [CrossRef]
21. Paulsen, E.; Barrios, S.; Lema, P. Ready-to-eat cherry tomatoes: Passive modified atmosphere packaging conditions for shelf life extension. *Food Packag. Shelf Life* **2019**, *22*, 100407. [CrossRef]
22. Ding, P.; Mashah, N.C. Growth, maturation and ripening of underutilized *Carissa congesta* fruit. *Fruits* **2016**, *71*, 171–176. [CrossRef]
23. Nagata, M.; Yamashita, I. A simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Nippon Shokuhin Kogyo Gakkaishi* **1992**, *39*, 925–928. [CrossRef]
24. Addai, Z.R.; Abdullah, A.; Mutalib, S.A. Influence of ripening stages on antioxidant properties of papaya fruit (*Carica papaya* L.). In Proceedings of the AIP Conference American Institute of Physics, Timisoara, Romania, 21–24 November 2013; Volume 1571, pp. 696–701.
25. Musa, K.H.; Abdullah, A.; Jusoh, K.; Subramaniam, V. Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): Effect of extraction techniques and solvents. *Food Anal. Methods* **2011**, *4*, 100–107. [CrossRef]
26. Li, S.M.; Hua, G.G.; Liu, H.X.; Guo, J.H. Analysis of defense enzymes induced by antagonistic bacterium Bacillus subtilis strain AR12 towards Ralstonia solanacearum in tomato. *Ann. Microbiol.* **2008**, *58*, 573–578. [CrossRef]
27. Soares, A.G.; Trugo, L.C.; Botrel, N.; da Silva Souza, L.F. Reduction of internal browning of pineapple fruit (*Ananas comusus* L.) by preharvest soil application of potassium. *Postharvest Biol. Technol.* **2005**, *35*, 201–207. [CrossRef]
28. Rivero, R.M.; Ruiz, J.M.; Romero, L. Can grafting in tomato plants strengthen resistance to thermal stress. *J. Sci. Food Agric.* **2003**, *83*, 1315–1319. [CrossRef]
29. Safari, Z.S.; Ding, P.; Zahidi, N.M.; Atif, A.; Wafa, S.; Aziz, A.; Yusoff, S.F. Maintenance of defence enzymes activities in tomato fruit during storage by chitosan and vanillin coating. *Int. J. Appl. Sci. Res.* **2021**, *4*, 177–181.

30. SAS Institute. *SAS 9.4 Output Delivery System: User's Guide*; SAS Institute: Cary, NC, USA, 2014.
31. Madani, B.; Mirshekari, A.; Sofo, A.; Tengku Muda Mohamed, M. Preharvest calcium applications improve the postharvest quality of papaya fruits (*Carica papaya* L. cv. Eksotika II). *J. Plant Nutr.* **2016**, *39*, 1483–1492. [CrossRef]
32. Senevirathna, P.A.W.A.N.K.; Daundasekera, W.A.M. Effect of postharvest calcium chloride vacuum infiltration on the shelf life and quality of tomato (cv. 'Thilina'). *Ceylon J. Sci. (Biol. Sci.)* **2010**, *39*, 35–44. [CrossRef]
33. Njoroge, C.K.; Kerbel, E.L.; Briskin, D.P. Effect of calcium and calmodulin antagonists on ethylene biosynthesis in tomato fruits. *J. Sci. Food Agric.* **1998**, *76*, 209–214. [CrossRef]
34. Agustí, M.; Juan, M.; Martínez-Fuentes, A.M.P.A.R.O.; Mesejo, C.; Almela, V. Calcium nitrate delays climacteric of persimmon fruit. *Ann. Appl. Biol.* **2004**, *144*, 65–69. [CrossRef]
35. Tzoutzoukou, C.G.; Bouranis, D.L. Effect of preharvest application of calcium on the postharvest physiology of apricot fruit. *J. Plant Nutr.* **1997**, *20*, 295–309. [CrossRef]
36. Sweetlove, L.J.; Williams, T.C.; Cheung, C.M.; Ratcliffe, R.G. Modeling metabolic CO2 evolution–a fresh perspective on respiration. *Plant Cell Environ.* **2013**, *36*, 1631–1640. [CrossRef]
37. Chepngeno, J.; Owino, W.O.; Kinyuru, J.; Nenguwo, N. Effect of calcium chloride and hydro cooling on postharvest quality of selected vegetables. *J. Food Res.* **2016**, *5*, 23. [CrossRef]
38. Pinzón-Gómez, L.P.; Deaquiz, Y.A.; Álvarez-Herrera, J.G. Postharvest behavior of tamarillo (*Solanum betaceum* Cav.) treated with $CaCl_2$ under different storage temperatures. *Agron. Colomb.* **2014**, *32*, 238–245. [CrossRef]
39. Ahmed, M.; Tariq, M. Enhancing postharvest storage life of tomato (*Lycopersicon esculentum* Mill.) cv. Rio Grandi using calcium chloride. *Am.-Eurasian J. Agric. Environ. Sci.* **2014**, *14*, 143–149.
40. Torre, S.; Borochov, A.; Halevy, A.H. Calcium regulation of senescence in rose petals. *Physiol. Plant.* **1999**, *107*, 214–219. [CrossRef]
41. Ranjbar, S.; Rahemi, M.; Ramezanian, A. Comparison of nano-calcium and calcium chloride spray on postharvest quality and cell wall enzymes activity in apple cv. Red Delicious. *Sci. Hortic.* **2018**, *240*, 57–64. [CrossRef]
42. Chuni, S.H.; Awang, Y.; Mohamed, M.T. Cell wall enzymes activities and quality of calcium treated fresh-cut red flesh dragon fruit (*Hylocereus polyrhizus*). *Int. J. Agric. Biol.* **2010**, *12*, 713–718.
43. Babu, I.; Ali, M.A.; Shamim, F.; Yasmin, Z.; Asghar, M.; Khan, A.R. Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. *Int. J. Adv. Res.* **2015**, *3*, 602–610.
44. Carvalho, P.D.T.; Clemente, E. The influence of the broccoli (*Brassica oleracea* var. itálica) fills weight on postharvest quality. *Food Sci. Technol.* **2004**, *24*, 646–651. [CrossRef]
45. Rahman, M.S.; Al-rizeiqi, M.H.; Guizani, N. Stability of vitamin C in fresh and freeze-dried capsicum stored at different temperatures. *J. Food Sci. Technol.* **2015**, *52*, 1691–1697. [CrossRef] [PubMed]
46. Arthur, E.; Oduro, I.; Kumah, P. Effect of maturity stage and postharvest calcium chloride treatment on the quality and storage life of tomatoes (*Lycopersicon esculentum* Mill.). *J. Postharvest Technol.* **2015**, *3*, 074–081.
47. Hussain, P.R.; Meena, R.S.; Dar, M.A.; Wani, A.M. Effect of postharvest calcium chloride dip treatment and gamma irradiation on storage quality and shelf-life extension of Red delicious apple. *J. Food Sci. Technol.* **2012**, *49*, 415–426. [CrossRef] [PubMed]
48. Devkota, P.; Devkota, P.; Khadka, R.; Gaire, K.R.; Dhital, P.R. Effects of chemical additives on shelf life of tomato (*Solanum lycopersicum*) during storage. *J. Agric. For. Univ.* **2019**, *3*, 69.
49. Abebe, Z.; Tola, Y.B. Effects of edible coating materials and stages of maturity at harvest on storage life and quality of tomato (*Lycopersicon Esculentum* Mill.) fruits. *Afr. J. Agric. Res.* **2017**, *12*, 550–565.
50. Wills, R.; Tirmazi, S.I.H.; Scott, K.J. Use of calcium to delay ripening of tomatoes. *HortScience* **1977**, *12*, 551–552.
51. Kong, K.W.; Khoo, H.E.; Prasad, K.N.; Ismail, A.; Tan, C.P.; Rajab, N.F. Revealing the power of the natural red pigment lycopene. *Molecules* **2010**, *15*, 959–987. [CrossRef]
52. Mujtaba, A.; Masud, T.; Butt, S.J.; Qazalbash, M.A.; Fareed, W.; Shahid, A. Potential role of calcium chloride, potassium permanganate, and boric acid on quality maintenance of tomato cv. Rio grandi at ambient temperature. *Int. J. Biosci.* **2014**, *5*, 9–20.
53. Bagheri, M.; Esna-Ashari, M.; Ershadi, A. Effect of postharvest calcium chloride treatment on the storage life and quality of persimmon fruits (*Diospyros kaki Thunb.*) cv.'Karaj'. *Int. J. Hortic. Sci. Technol.* **2015**, *2*, 15–26.
54. Cano, A.; Acosta, M.; Arnao, M.B. Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (*Lycopersicon esculentum* Mill.). *Postharvest Biol. Technol.* **2003**, *28*, 59–65. [CrossRef]
55. Chandra, S.; Chakraborty, N.; Chakraborty, A.; Rai, R.; Bera, B.; Acharya, K. Abiotic elicitor-mediated improvement of innate immunity in *Camellia sinensis*. *J. Plant Growth Regul.* **2014**, *33*, 849–859. [CrossRef]
56. Ma, F.; Lu, R.; Liu, H.; Shi, B.; Zhang, J.; Tan, M.; Jiang, M. Nitric oxide-activated calcium/calmodulin-dependent protein kinase regulates the abscisic acid-induced antioxidant defense in maize. *J. Exp. Bot.* **2012**, *63*, 4835–4847. [CrossRef] [PubMed]
57. Tejeswini, M.G.; Sowmya, H.V.; Swarnalatha, S.P.; Negi, P.S. Antifungal activity of essential oils and their combinations in vitro and in vivo conditions. *Arch. Phytopathol. Plant Prot.* **2014**, *47*, 564–570. [CrossRef]
58. Chandra, S.; Chakraborty, N.; Chakraborty, A.; Rai, R.; Bera, B.; Acharya, K. Induction of defense response against blister blight by calcium chloride in tea. *Arch. Phytopathol. Plant Prot.* **2014**, *47*, 2400–2409. [CrossRef]
59. Chéour, F.; Souiden, Y. Calcium delays the postharvest ripening and related membrane-lipid changes of tomato. *J. Nutr. Food Sci.* **2015**, *5*, 1.
60. Chakraborty, N.; Chandra, S.; Acharya, K. Biochemical basis of improvement of defense in tomato plant against Fusarium wilt by $CaCl_2$. *Physiol. Mol. Biol. Plants* **2017**, *23*, 581–596. [CrossRef]

61. Chandra, S.; Chakraborty, N.; Dasgupta, A.; Sarkar, J.; Panda, K.; Acharya, K. Chitosan nanoparticles: A positive modulator of innate immune responses in plants. *Sci. Rep.* **2015**, *5*, 1–14. [CrossRef]
62. Parmar, P.; Subramanian, R. Biochemical Alteration Induced in Tomato (*Lycopersicum esculentum*) in Response to *Fusarium oxysporum* f. sp. *lycopersici*. *Afr. J. Basic Appl. Sci.* **2012**, *4*, 186–191.
63. Tian, S.; Wan, Y.; Qin, G.; Xu, Y. Induction of defense responses against Alternaria rot by different elicitors in harvested pear fruit. *Appl. Microbiol. Biotechnol.* **2006**, *70*, 729–734. [CrossRef]
64. Gupta, N.S.; Banerjee, M.; Basu, S.K.; Acharya, K. Involvement of nitric oxide signal in '*Alternaria alternata*' toxin-induced defense response in '*Rauvolfia serpentina*' Benth. ex Kurz calli. *Plant Omics J.* **2013**, *6*, 157–164.
65. Ruiz-García, Y.; Gómez-Plaza, E. Elicitors: A tool for improving fruit phenolic content. *Agriculture* **2013**, *3*, 33–52. [CrossRef]
66. Sinha, S.R.; Singha, A.; Faruquee, M.; Jiku, M.A.S.; Rahaman, M.A.; Alam, M.A.; Kader, M.A. Postharvest assessment of fruit quality and shelf life of two elite tomato varieties cultivated in Bangladesh. *Bull. Natl. Res. Cent.* **2019**, *43*, 1–12. [CrossRef]
67. Sohail, M.; Ayub, M.; Khalil, S.A.; Zeb, A.; Ullah, F.; Afridi, S.R.; Ullah, R. Effect of calcium chloride treatment on postharvest quality of peach fruit during cold storage. *Int. Food Res. J.* **2015**, *22*, 2225–2229.
68. Gharezi, M.; Joshi, N.; Sadeghian, E. Effect of postharvest treatment on stored cherry tomatoes. *J. Nutr. Food Sci.* **2012**, *2*, 1–10.