



Article

Extending Shelf Life and Maintaining Quality of Tomato Fruit by Calcium Chloride, Hydrogen Peroxide, Chitosan, and Ozonated Water

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Abstract: Tomatoes are perishable fruit that makes them deteriorate rapidly during the post-harvest chain. Therefore, the effect of calcium chloride (CaCl₂), chitosan, hydrogen peroxide (H₂O₂), and ozonated water on the storage ability and quality of tomato fruit (*Solanum lycopersicum* L. cv. 448) stored at 10 °C for 28 d was studied. Weight loss, firmness, fruit color, total soluble solids (TSS), titratable acidity, total carotenoids, and ascorbic acid content (AsA) of treated tomato fruit were recorded. Our results revealed that all tested treatments significantly extended the shelf-life and maintained quality of tomato fruit compared to the control. Chitosan and CaCl₂ were the most effective treatments in maintaining quality attributes. Furthermore, a correlation study suggested that AsA and total carotenoids played a vital role in conserving tomato fruit quality during storage. PC1 had strong positive loading for pH, appearance, firmness, AsA, TSS, carotene, fruit color (L* & b*) and a strong negative loading for lycopene content, color (a), weight loss, and color index. PC2 had high positive loading for total acidity and total sugar content.

Keywords: *Solanum lycopersicum*; antioxidants; storage; quality; postharvest



Citation: Shehata, S.A.; Abdelrahman, S.Z.; Megahed, M.M.A.; Abdeldaym, E.A.; El-Mogy, M.M.; Abdelgawad, K.F. Extending Shelf Life and Maintaining Quality of Tomato Fruit by Calcium Chloride, Hydrogen Peroxide, Chitosan, and Ozonated Water. *Horticulturae* **2021**, *7*, 309. <https://doi.org/10.3390/horticulturae7090309>

Academic Editor: Tianbao Yang

Received: 19 July 2021

Accepted: 10 September 2021

Published: 14 September 2021

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is considered one of the most important vegetable crops worldwide for fresh market and processed products due to its health and economic importance [1]. It has been reported that tomato fruit have a considerable value of the most important antioxidants such as lycopene, carotenoids, vitamin C, and minerals, which can play a vital role for suppressing the development of some human diseases including prostate, colon, and breast cancers [2]. Additionally, consumption about 100 g of tomato can supply the human body with 40% of the recommended daily dosage of vitamin C which can enhance the immune system, lower blood pressure and cholesterol [3]. Furthermore, tomatoes are classified as a climacteric fruit and deteriorate rapidly after harvest due to soft textures and its susceptibility to microbial infection. Tomatoes are harvested at various maturity stages, including green mature, breaker, turning, light red, and full red. The most preferred stages for consumers are the light and full red stages, which decay vastly after harvest. Fruit and vegetables quality is mainly affected by postharvest conditions such as transportation and storage conditions [4]. Some previous postharvest application has been utilized to extend the shelf-life of tomatoes during storage such as using hydrogen sulfide [5], chitosan coating [6], abscisic acid [7], and the use of essential oils [8]. Previous studies showed that CaCl₂ reduced the fruit decay ratio and improved the hardness of

tissues and cell walls [9]. Chitosan is inexpensive and a natural compound which is obtained from chitin. Edible coating of chitosan alone or combined with essential oils provides an excellent antimicrobial agent, minimizing the fruit respiration and water loss rates [10,11]. Furthermore, the efficiency of oxidative substances, including ozone and H_2O_2 on stored vegetables and fruit, was mentioned previously in few reports [12,13]. However, to our knowledge, the effect of exogenous application of H_2O_2 and ozonated water on postharvest behavior of tomato fruit has not been reported before. Therefore, the aim of this study was to evaluate the effect of chitosan, $CaCl_2$, H_2O_2 , and ozonated water as postharvest treatments on maintaining postharvest quality and prolonging the shelf life of tomato fruit during refrigerated storage.

2. Materials and Methods

2.1. Experimental Design and Treatments

Fruit of tomato hybrid cv. 448 (Syngenta company for seeds) were harvested at the breaker stage from a private farm (Abo Shalaby) in Ismailia Governorate, Egypt (30.5831° N, 32.2654° E). The tomato fruit were transported within one hour to the postharvest laboratory of the Vegetable Department, Faculty of Agriculture, Cairo University. Fruit free from any defects, diseases, unripe/immature, and uniform in size and weight (150 g, round type and large size) were chosen for the experiments. Selected tomato fruits were washed with tap water and then immersed in the following different solutions for 5 min. at room temperature: chitosan (0.5%) [10], calcium chloride (1%) [9], hydrogen peroxide (0.12%) [13], and ozonated water (1%) [12]. The control group was left without any treatments. The treated fruit were then left until fully dried at room temperature, packed in polystyrene trays (40 cm × 20 cm), and then covered by polyethylene sheet. Each tray had about 450 g (three fruit) of tomato fruit. The trays were stored for 28 days at 10 °C plus 2 days at 20 °C and 90–95% relative humidity (RH) in complete darkness. All measured parameters were recorded at time intervals of 0, 7, 14, 21, and 28 d. after applying treatments. The experiment was repeated twice with three replicates for each one and the averages were used.

2.2. Preparation Treatments Solutions

Chitosan 0.5% (*w:v*) (Qualikems, Vadodara, India) was dissolved in acetic acid 1% (*v:v*) (El-Nasr Company, Helwan, Egypt) then stirred using a magnetic stirrer for 6 h. in accordance to Xiao et al. [14]. Ozonated water was provided by an O_3 generator 101 (Cosemar Ozono, Madrid, Spain). Calcium chloride (Loba Chemie, Mumbai, India) was prepared at 1% by dissolving 1 g in 100 mL distilled water. Hydrogen peroxide 0.12% (*v:v*) was prepared by mixing 0.12 mL of hydrogen peroxide (Qualikems, Vadodara, India) in 100 mL distilled water in a homogenizer.

2.3. Appearance, Weight Loss, Color Parameters, and Firmness Determination

Appearance was evaluated using a scale from 9 to 1, where 9 = excellent, 7 = good (minor defect), 5 = fair (slightly to moderately objectionable defects), 3 = poor (excessive defect), 1 = unacceptable (extremely defect). Samples were submitted to six professional panelists from the vegetables department, Faculty of Agriculture, Cairo University, Giza, Egypt, for general appearance evaluation. Fruit rated 3 or below were considered as unmarketable. It was recorded for both the shriveling, wilting, color, change, and decay or any visible deterioration as described by Ali et al. [15]. Treated fruit were weighed after drying and at every sampling time to measure weight loss percentage as described by Awad et al. [16]. Fruit color was determined using a Chroma meter CR-400 (Konica Minolta, INC, Tokyo, Japan) for the estimation of L^* (lightness), a^* (change color from red to green), and b^* (change color from yellow to blue). The color index (CI) was calculated according to Voss [17]. Fruit firmness was measured using a digital food pressure tester (Force Gauge Model M4-200 MARK) with a 2 mm diameter flat probe. Five fruits per

treatment were used to measure firmness at three points on the equatorial region. The results were expressed in $\text{kg}\cdot\text{cm}^{-2}$.

2.4. Total Soluble Solids (TSS), Ascorbic Acid (AsA), and Titratable Acidity (TA) Determination

Total soluble solids (TSS) were evaluated using a digital refractometer (model PAL-1, Atago, Tokyo, Japan) and the values of TSS were expressed as °Brix. Ascorbic acid of tomato samples was determined using titration methods [18]. Briefly, ten gram of tomato fruit tissue was mixed with 90 mL of oxalic acid (6%) (Nice Chemical Ltd., Kochi, India). The sample was then filtered using a filter paper, 25 mL of filtrated solution was titrated by 2,6-dichlorophenol indophenol (Loba Chemie, Mumbai, India). The values were reported as mg/g fresh weight (FW).

The percentage of titratable acidity (TA) in tomato samples was determined by using 5 g of treated tomato fruit and homogenized with 50 mL of distilled water, then filtered. The aliquot was titrated with 0.1 N NaOH (El-Nasr Company, Giza, Egypt) using phenolphthalein as an indicator [19]. The values were reported as the percentage of citric acid, according to the following formula:

$$\text{Acidity}\% = [(\text{Titre value} \times \text{Normality} \times \text{m.eq.wt. of acid}) / (\text{Volume of sample})] \times 100.$$

2.5. Total Sugar, Lycopene, and Carotenoid Contents Determination

Total sugar content was determined using the phenol-sulphuric acid (El-Nasr Company, Giza, Egypt) according to the method described by Shehata et al. [11]. Lycopene and carotenoid contents were measured according to the method described by Abdelgawad et al. [1]. Tomato samples were homogenized and one gram was mixed with a 10 mL mixture of Acetone-Hexane (4:6 v:v Merk, Darmstadt, Germany). The solution was then left to separate into distinct and non-polar layers. The spectrophotometer (Unico, UV2000, Rochester, NY, USA) was used to measurement of absorbance at 663, 645, 505, and 453 nm, and then lycopene and carotenoid content calculated according to the following equations:

$$\text{Lycopene} = -0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453},$$

$$\text{B-carotene} = 0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}.$$

2.6. Statistical Analysis

The data of experiments were subjected to the two-way analysis of variance (ANOVA) using the M.Stat software (Version 2.1, Michigan State University, East Lansing, MI, USA). Means of different treatments were compared by the Duncan test at 5% (LSD). The principal component analysis of the different applied solutions and physiochemical properties of tomato fruit, during the storage periods, were performed and displayed on the correlation heat map.

3. Results and Discussion

3.1. Appearance, Weight Loss, Color Parameters, and Firmness

Appearance was influenced by storage periods and treatments whereas the interaction was not significant (Table 1). As shown in Figure 1A, appearance of tomato fruit significantly reduced by increasing the storage period. Similar findings were confirmed by previous study [11]. They found that general appearance of strawberries decreased with increasing of storage period. The decrease in appearance during storage could be due to wilting, shriveling, color change, and deterioration [3]. All treatments significantly delayed the deterioration in fruit appearance (shriveling and color deterioration) during storage periods (Figure 1B). Both CaCl_2 , and H_2O_2 treatments showed better appearance than other treatments or the control. Additionally, the difference between ozonated water treatment and the control was not significant. Previous study has shown that chitosan coating improves the external appearance and visual quality of fruits and vegetables, which

is consistent with our findings [20]. Additionally, CaCl_2 application conserved the appearance during storage [9,21]. The positive effect of chitosan could be attributed to its ability in reducing the respiration rate, moisture loss, ethylene production, delay product ripening, and inhibit the growth and development of undesirable microbes [22]. The beneficial effects of chitosan in conserving the appearance of tomato fruit during refrigerated storage could be also due to the reduction of lycopene degradation by chitosan treatment [20]. A strong negative correlation between appearance and weight loss was observed (Table 2).

Table 1. Analysis of variance (mean square) of tomato fruit attributes (appearance, weight loss, firmness, TSS and pH) stored at 10 °C for 28 days + 2 day at 20 °C and 90–95% RH.

Source	Appearance	Weight Loss (%)	Firmness (Kg/cm)	TSS	pH
Storage period (S)	57.42 ***	1973.2 ***	14.03 ***	6.87 ***	1.09 ***
Treatment (T)	1.48 **	110.29 ***	4.14 ***	6.94 ***	1.88 ***
S × T	0.703 ns	14.26 **	0.147 ns	1.08 ***	0.106 ns

ns, **, *** not significant or significant at, $p \leq 0.01$, $p \leq 0.001$, analysis of variance.

Table 2. Pearson's correlation analysis between the physicochemical properties of tomato fruit.

Variables	Weight Loss	Appearance	Firmness	TSS	L*	a*	b*	pH	TA	AsA	Lycopene	Carotene	CI
Appearance	-0.91												
Firmness	-0.90	0.90											
TSS	-0.09	-0.14	0.13										
L*	-0.38	0.53	0.55	-0.21									
a*	0.78	-0.72	-0.82	-0.11	-0.29								
b*	-0.36	0.32	0.45	0.11	0.59	-0.31							
pH	-0.36	0.49	0.33	-0.36	-0.08	-0.42	-0.10						
TA	0.16	-0.47	-0.20	0.80	-0.35	0.04	0.02	-0.53					
VC	-0.80	0.72	0.85	0.23	0.54	-0.84	0.53	0.17	0.06				
Lycopene	0.85	-0.72	-0.88	-0.38	-0.33	0.83	-0.49	-0.16	-0.15	-0.88			
Carotene	-0.20	0.11	0.37	0.32	0.41	-0.49	0.36	-0.23	0.37	0.66	-0.44		
CI	0.73	-0.76	-0.85	0.00	-0.69	0.86	-0.57	-0.28	0.16	-0.86	0.76	-0.57	
Sugars	0.33	-0.57	-0.41	0.70	-0.54	0.22	-0.25	-0.51	0.89	-0.15	0.05	0.21	0.41

Values in bold are different from 0 with a significance level alpha = 0.05.

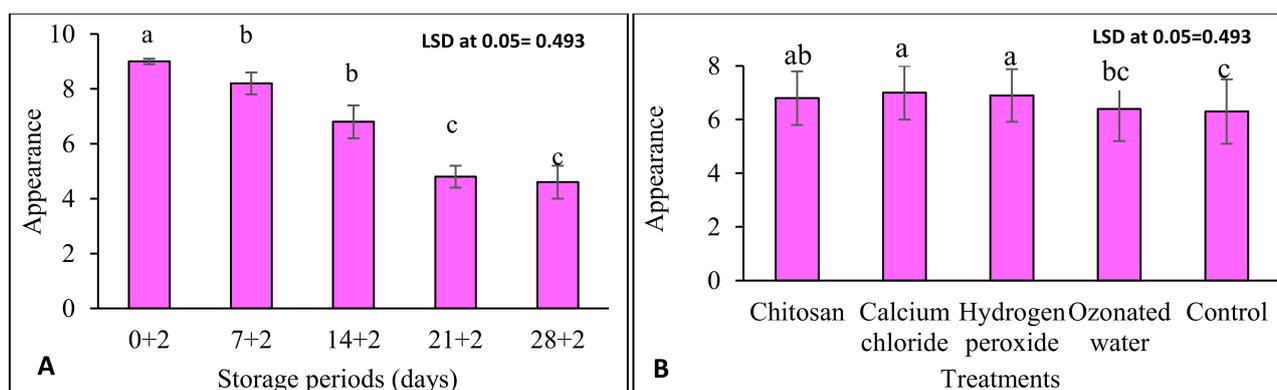


Figure 1. Appearance of tomato fruit as affected by storage periods (A) and treatments (B).

Weight loss is the most important factor for the quality and shelf-life of horticulture crops [18]. As shown in Table 1 and supplementary Table S1, weight loss, was affected by storage period, treatments, and their interactions. As expected, the weight loss increased during the storage period for all treatments (Figure 2A). After 7 + 2 days of storage and shelf-life until the end of storage, the percentage of weight loss of tomato fruit treated with CaCl_2 , chitosan, ozonated water, and H_2O_2 was significantly ($p < 0.05$) lower than non-treated fruit (control) as well as reduced progress of ripening. The lowest value of weight loss was observed in chitosan treatment compared to all other treatments.

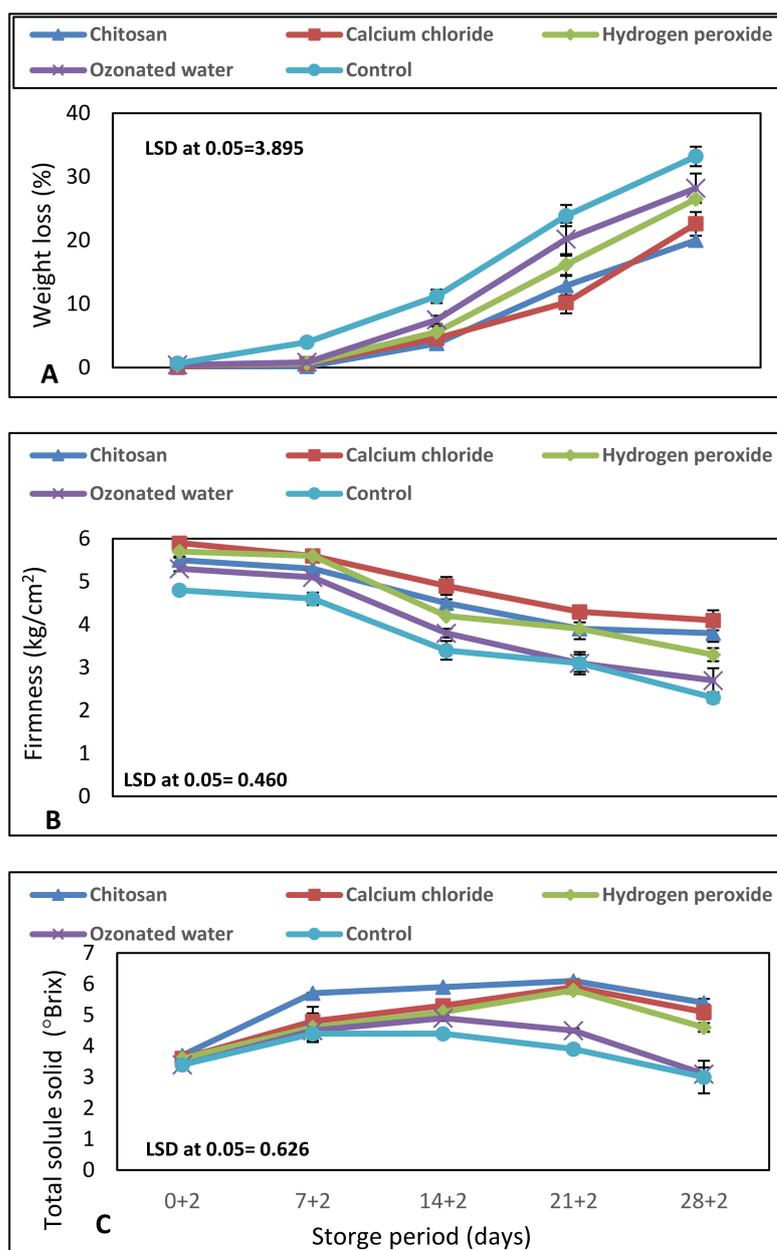


Figure 2. Weight loss% (A), firmness (B), and total soluble solid (°Brix) (C) of treated tomato fruit as affected by the interaction between the treatments and storage periods.

High relative air humidity and low temperature were employed to decrease postharvest water loss [23]. Thus, preventing water loss which could increase shelf-life of the fresh products. Weight loss was increased with increasing storage periods in all treatments and the control (Figure 2A). The high weight loss percentage at the end of storage period was related to the two days at shelf-life at 20 °C. It has been reported that water loss from fresh products causes unfavorable metabolic changes in plant cells which activate the enzymes. These enzymatic activities accelerate senescence and decrease the nutritional values [24]. Thus, the use of edible coating such as chitosan could help to prevent senescence and extend shelf-life. It is clear from current and previous reports that coating tomatoes with chitosan reduces weight loss when compared to the control fruit, most likely as a result of covering the cuticles on the fruit surfaces with chitosan [20]. The positive role of chitosan application in reducing weight loss of tomato fruit could be due to its ability to form a thin semipermeable film on the outer surface of the fruit which reduces transpiration [25].

Some previous studies have found that chitosan is effective for delaying weight loss in other commodities such as strawberries [26] and cucumber [27] which is in agreement with our results.

Moreover, the reduction in weight loss by CaCl_2 treatment could be due to the role of calcium in the creation of calcium pectate hydrogel, which holds more water and slows the dehydration process [28]. In our study, ozonated water treatment reduced weight loss compared to the control. This result is in agreement with Rodini et al. [29] who found that ozone treatment reduced weight loss of tomato fruit during storage at 20 °C for 9 d. Ozonated water treatment could reduce weight loss from tomato fruit by maintaining the cell wall and reducing pectin solubilization [30].

Firmness is considered one of the most important indicators of tomato quality [31]. Maturation process of tomato fruit affects the firmness and the composition of the cell wall polysaccharides [32]. In this study and previous studies, firmness of all tomato samples decreased with increasing storage time (Figure 2B and supplementary Table S1). This reduction in firmness is mainly due to the activity of some endogenous enzymes related to cell wall degradation [33]. Furthermore, softening changes have been linked to the degradation of the middle lamella of cortical parenchyma cells, which results in a significant increase in pectin solubilization [34]. During the entire storage period, the tomato fruit treated with chitosan and CaCl_2 were firmer than other treatments and the control. Chitosan and CaCl_2 treatments showed reduced ripening of fruits compared to other treatments and the control. Calcium is a key cation in plant nutrients which is required for maintaining firmness of the cell wall and middle lamella. It has been reported that application of exogenous calcium to fruit tissue maintains the structure of the cell wall and middle lamella which conserves firmness during cold storage [35]. In this study and previously, application of chitosan can also prevent the loss of tomatoes firmness under cold storage conditions [36]. This result might be due to the role of chitosan in reducing oxygen availability in tissue which reduces the activity of the enzymes responsible for firmness loss such as pectin-esterase and polygalacturonase resulting in higher firmness during storage [37]. It has been well known that H_2O_2 is an economical, safe, and sanitizing agent used for fruit and vegetables [38]. A strong negative correlation was recorded between firmness and the other parameters including a^* , lycopene content, and color index. On the other hand, a positive correlation was found between firmness and L^* , b^* , and vitamin C (Table 2).

3.2. Total Soluble Solids and pH

Total soluble solids were gradually increased in chitosan, calcium chloride, and hydrogen peroxide treatments with extended storage period until 21 + 2 days of storage plus shelf-life, and then decreased until the end of the storage period (Figure 2C and supplementary Table S1). On the other hand, total soluble solids in ozonated water and control treatments were increased until the 14 + 2 days of storage period and then gradually decreased until the end of storage period. The increment in TSS might be due to the solubilization of cellulose and hemi-cellulose in cell wall [39] or water loss [18]. The decline of TSS at the end of the storage period could be due to the use of sugar in the respiratory process which decreases the level of TSS in all treatments and the control [40].

At the last two periods of storage, TSS values of chitosan, CaCl_2 , and H_2O_2 treated fruit were higher than control and other treatments. Total soluble solids consist of some compounds such as sugar, vitamins, and acids which are mainly used for the respiration process. Previous work and our study indicated that chitosan application reduces the loss of TSS during storage [41]. It has been reported that TSS of tomatoes were increased and then decreased during the cold storage, which is in agreement with the results of this study [42]. Chitosan could preserve TSS by reducing the respiration process during cold storage [31]. It is reported that CaCl_2 treatment reduce respiration process [28]. As a result, the amount of TSS which is used for respiration in fruit treated with CaCl_2 is lower than untreated fruit. In this study, hydrogen peroxide was used as a disinfectant and it decrease

the loss of TSS during storage. This result is in agreement with previous work where H₂O₂ treatment also delayed the decline of TSS in muskmelon fruit [43]. In this study, positive correlation was noted between TSS and TA or total sugar (Table 2).

As shown in Figure 3A, the pH of tomato juice progressively decreased with increasing storage period. This result is in agreement with previous study on tomatoes. They noted a decrease in pH values until 21 days of refrigerated storage at 10 °C [34]. Figure 3B shows that a minimum value of pH was recorded in fruit treated with chitosan and CaCl₂. Negative correlation between pH and both TA and total sugar was observed (Table 2).

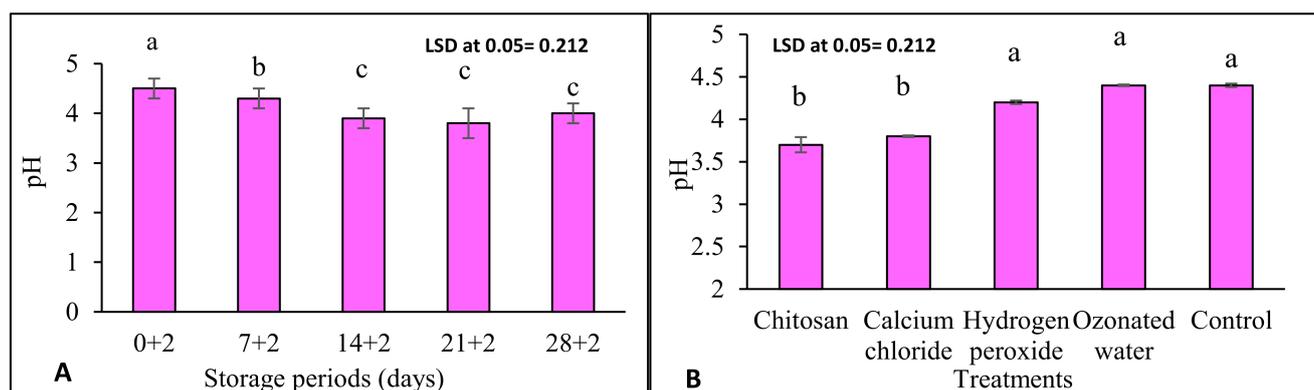


Figure 3. pH of tomato fruit as affected by storage periods (A) and treatments (B).

3.3. Surface Color Evaluation

Color is one of the major visual quality parameters that effects consumer acceptance of fresh products including tomato fruit. All color parameters L* (lightness), a* (green/red), b* (yellow/blue), and color index (CI) were significantly affected by the storage period, treatment, and their interaction (Table 3).

Table 3. Analysis of variance (mean square) of tomato fruit surface color.

Source	L*	a*	b*	Color Index
Storage period (S)	229.70 ***	741.40 ***	144.44 ***	1290.73 ***
Treatment (T)	68.32 ***	337.33 ***	44.19 ***	550.22 ***
S × T	36.84 ***	18.94 ***	29.57 ***	51.28 ***

*** significant at $p \leq 0.001$, analysis of variance.

The lightness (L*) of tomatoes fruit is one of the most important colorimetric parameters that is strongly affected during storage. The values of L* and b* of tomato fruit significantly decreased with increasing storage period, showing darker and less yellowing of fruits (Figure 4A,C and supplementary Table S1). This result is agreeing with Breda et al.'s study [24]. The reduction of L* during cold storage could be related to the surface dehydration which decrease surface glossiness [44]. Red color is one of the most attractive visual parameter to consumers [45]. Red color is gradually developed during tomato fruit ripening and maturation due to the degradation of chlorophyll and formation of lycopene pigment [46]. The values of a* and CI increased with increasing the storage period, showing redder and mature fruit (Figure 4B,D and supplementary Table S1). Chitosan treatment was the most effective treatment for conserving higher L* and b* values and lower a* and CI values during the entire storage period. This result indicated that chitosan treatment retarded the ripening development (Figure 4). Our result is in agreement with previous works who indicated that chitosan treatment conserved higher L* and b* values [47,48] and a lower color (less red color) index during storage [49]. It was found that chitosan coating treatment reduced O₂ levels and increased CO₂ levels within tomato fruit which retarded ripening and color development [24,50]. As a result, the lower respiration rate can help to slow down the deterioration process and preserve fruit quality

after harvest. Additionally, application of chitosan as an edible coating on fruit surface could provide an additional gloss on fruit surface which increases and conserve the L^* during cold storage [51]. Positive correlation was found between L^* from one side and b^* , vitamin C, and carotene from other side (Table 2). Additionally, a negative correlation between L^* and both CI and total sugar was observed.

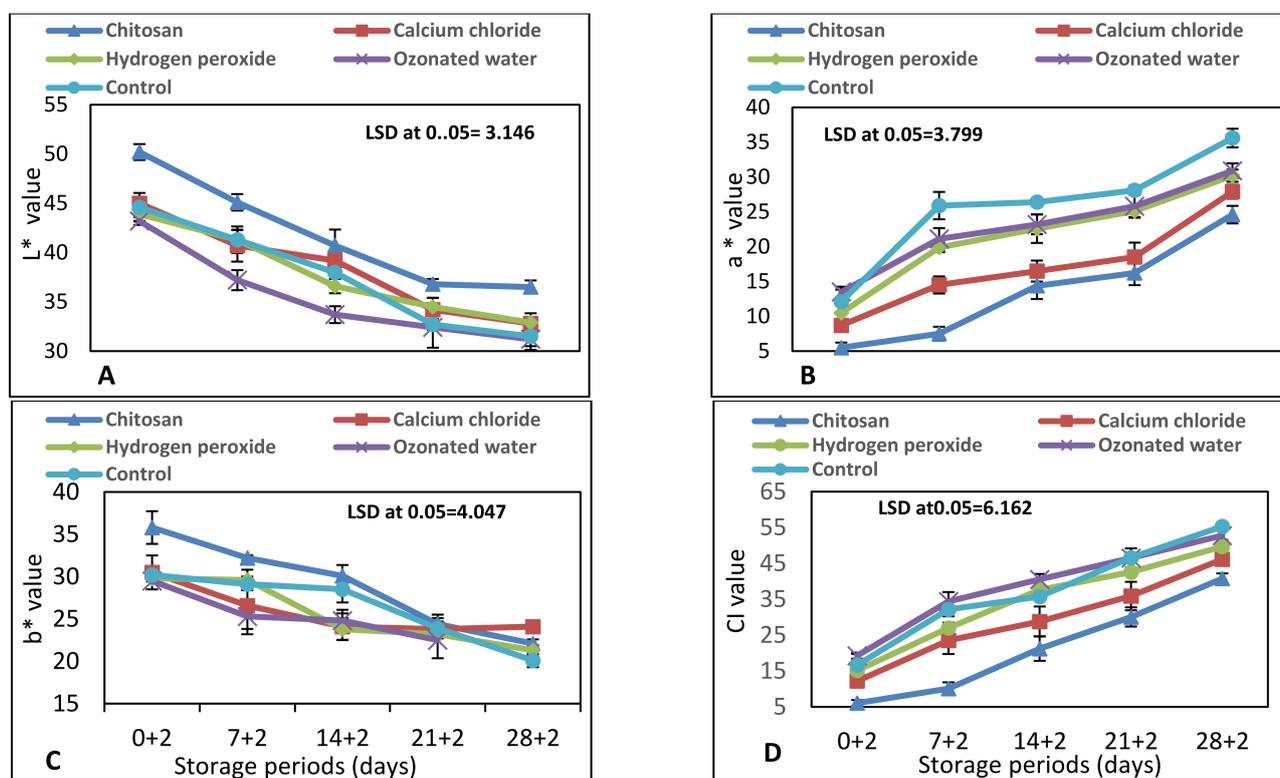


Figure 4. Values of L^* (A), a^* (B), b^* (C), and CI (D) of tomato fruit as affected by the interaction between the treatments and storage periods.

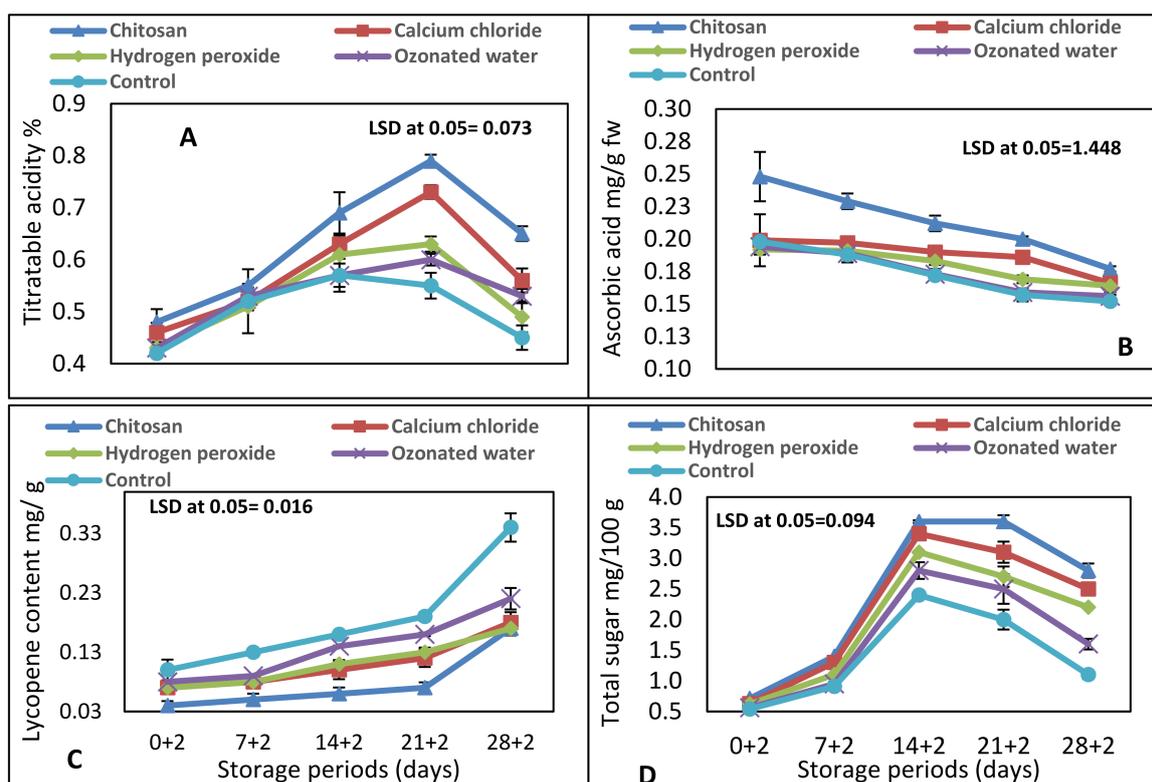
3.4. Titratable Acidity and Ascorbic Acid

Titrate acidity and AsA, significantly affected by treatment, storage period and their interaction (Table 4). As shown in Figure 5A and supplementary Table S1, the content of TA in all treatments and the control increased constantly with increasing the storage period until 21 days of storage then decreased which is in agreement with previous work [36]. This increase in TA at the beginning of storage could be due to the increase of Cl^- ion [52]. Moreover, the reduction in TA content after 21 + 2 of storage might be attributed to the use of titrate acids in the respiration process and its metabolism [53]. In contrast with our results, previous work reported that TA in tomatoes decreased with increasing storage time [29]. The differences with our results may be due to the fact that our TA measurement was carried out after two days at 20 °C as a shelf-life. Thus, the increase in TA was recorded due to the high temperature of shelf-life storage which enhances the accumulation of titrate acids [54]. Chitosan treatment slowed the loss of TA in tomato fruit during refrigerated storage which is in agreement with previous results on pear fruit [37] and pineapple [55]. This result may be due to reduction of respiration rate by chitosan coating [37] via changes in the internal O_2 and CO_2 composition of the tissue. Additionally, it was reported that $CaCl_2$ treatment reduces the respiration process [56]. Thus, the amount of TA which is used in respiration process in tomato fruit treated with $CaCl_2$ is lower than untreated fruit. A strong positive correlation between TA and total sugar was observed (Table 2).

Table 4. Analysis of variance (mean square) of tomato fruit chemical properties.

Source	Titrateable Acidity (%)	Ascorbic Acid (%)	Lycopene (mg/g)	Carotenoid (mg/g)	Sugar Content (mg/100 g)
Storage period (S)	0.104 ***	58.07 ***	0.046 ***	0.006 ns	17.213 ***
Treatment (T)	0.040 ***	46.95 ***	0.028 ***	0.542 ***	2.599 ***
S × T	0.004 **	2.77 ***	0.001 ***	0.007 ns	0.222 ***

ns, **, *** not significant or significant at $p \leq 0.01$, $p \leq 0.001$, analysis of variance

**Figure 5.** Titrateable acidity (A), ascorbic acid (B), lycopene (C), and total sugar contents (D) of tomato fruit as affected by the interaction between the treatments and storage period.

Ascorbic acid is the most important antioxidant compound present in tomato fruit [57]. Some pre and postharvest factors can affect AsA levels such as light during refrigerated storage [58]. They also noted that AsA content was dependent on the maturity stage. Ascorbic acid content significantly decreased with the increasing of storage period for all treatments and the control (Figure 5B and supplementary Table S1). This decrease could be due to the use of AsA in respiration process [37] or the oxidation of AsA [38]. Figure 5B shows that chitosan coating was the most effective treatment for reducing the loss of vitamin C during storage followed by CaCl₂ and H₂O₂, respectively. It has been reported that chitosan coating maintained AsA in tomatoes [48] and some other fruit during storage such as papaya [59]. This result could be due to the low O₂ permeability of the chitosan coating resulting in lower respiration process [27,48] which conserved AsA from the loss. A strong negative correlation between AsA and lycopene content and CI was observed (Table 2).

3.5. Lycopene, Total Sugar Contents, and Carotenoids Contents

Lycopene is the pigment mainly accountable for the distinguishing red color of ripe tomato fruit. As shown in Figure 5C and supplementary Table S1, lycopene content was increased with increasing storage time in all treatments and the control. However, the increase in lycopene content was lower in fruit treated with chitosan, CaCl₂, H₂O₂,

and ozonated water than the untreated fruit. This result could be attributed to the role of chitosan and CaCl_2 for reducing respiration and maturity process [27,55] leading to slowing down of fruit ripening and lycopene biosynthesis. Some previous reports are in accordance with our results [36,48], they found that chitosan treatment increased retention of lycopene content during refrigerated storage of tomato fruit.

One of the most basic factors for evaluating fruit ripening in the fruit is total sugars [36]. In this study, total sugar content in tomato fruit for all treatments increased with prolongation of the storage period until the 14th day of storage and then decreased until the end of storage (Figure 5D and supplementary Table S1). These findings were consistent with previous works, who found that the sugar content of tomatoes increased as they matured from pink stage to red ripen [60,61]. The increase in total sugar content in tomato fruit during the first period of storage might be explained by the moisture loss during storage [62]. The decline in total sugar content in tomato fruit after 14 d from storage might be due to the consumption of total sugar in respiration process during storage [36]. Chitosan and CaCl_2 treatments maintained the highest level of total sugar content in tomato fruit throughout storage period as compared to the control. The previously observed result might be related to the hypotheses that chitosan and CaCl_2 control respiration process and their related metabolic activities leading to an accumulation of sugar content [55,63]. In this study, tomato fruit treated with H_2O_2 , and ozonated water conserved higher total sugar content than the control.

Carotenoids have a dual role in tomato fruit. They act as antioxidants (thus alleviating oxidative stress) and they are of high value for human intake [64]. Chitosan and CaCl_2 treated fruit conserved significantly higher carotenoid content and delayed repining than the control and other treatments during storage period (Figure 6). The favorable effect of chitosan treatment on carotenoids retention until the end of refrigerated storage is consistent with previous studies on tomato [61,62]. This result may be related to the role of chitosan for reducing respiration rate which resulted in higher carotenoid content during storage. Furthermore, chitosan could preserve carotenoid from oxidation during storage [64]. CaCl_2 treatment conserved carotenoid content in tomato fruit during storage which could be due to its role for reducing respiration metabolism [55] resulting in higher carotenoid content. Negative correlation between lycopene and carotenoids content was observed while a strong positive correlation was recorded between lycopene and CI (Table 2).

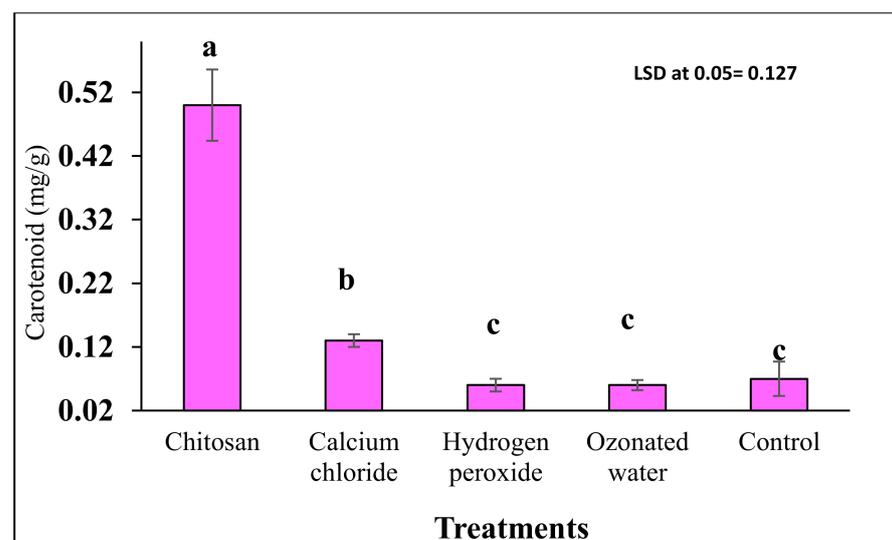


Figure 6. Carotenoid content ($\text{mg}\cdot\text{g}^{-1}$ fresh weight) of tomato fruit as affected by treatments.

4. Correlation Study

Principal component analysis (PCA), Pearson's correlation analysis, and Heatmap of the changes in physicochemical properties of tomato fruits during storage were presented in Table 2 and Figure 7. Considering the variances in the tomatoes quality, conserved at 10 °C for 28 days plus two days at 20 °C, treated with chitosan (CH) at 0.5%, calcium chloride (CaCl₂) at 1%, hydrogen peroxide (H₂O₂) at 0.12%, Ozonated water (O₃), 14 indexes of tomato fruits during storage periods were integrated using two-dimensional principal component analysis (PCA) with the SPSS 20.0 software. PCA was used to additional integrate and analyze the findings of postharvest quality indicators of tomato fruits. Principal components (PCs) denote 74.08% of the total variance of the data set. The contribution rate of PC1 and PC2 was 23.84% and 50.25% of the variance in the data set, respectively. PC1 had strong positive loading for pH, general appearance (appearance), firmness, AsA (V.C), TSS, carotene, fruit color (L* & b*) and a strong negative loading for lycopene content, color (a*), weight loss, and color index (CI).

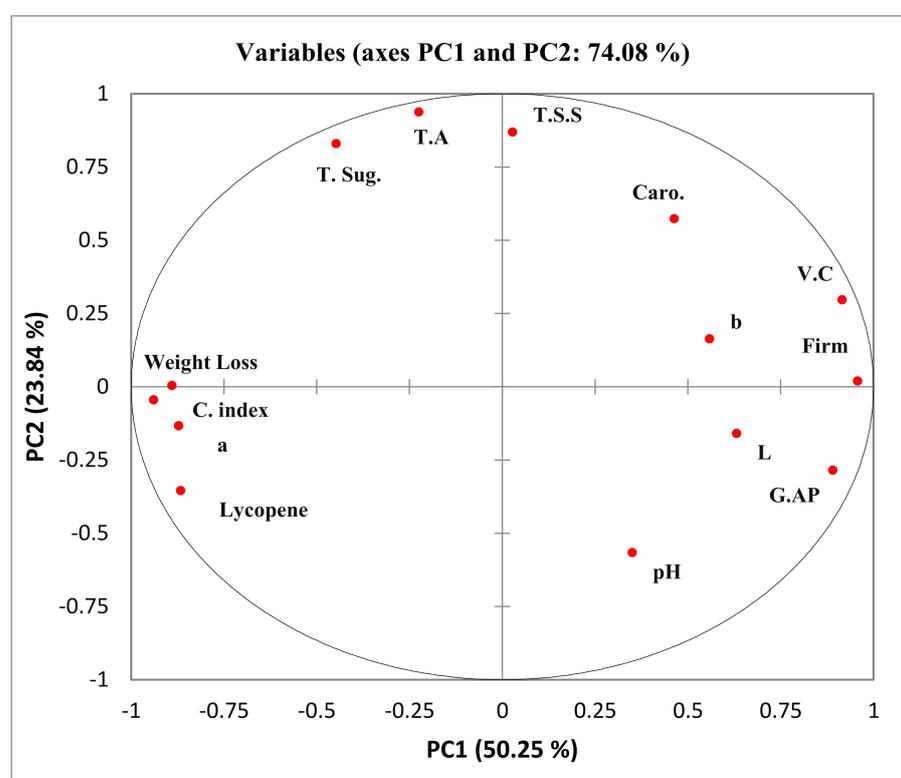


Figure 7. Principal component analysis of the main physicochemical quality of post-harvest tomato fruit.

PC2 had high positive loading for total acidity and total sugar content. Correlation-based method using the Pearson coefficient was used to observe the positive and negative correlations between the physicochemical parameters of treated and stored tomato fruits. Significant correlations (in bold number) and insignificant relationships (unbold number) are presented in Table 2. Pearson correlation analysis showed that a general appearance had a significant positive correlation with fruit firmness, L*, AsA, and total carotenoids. Meanwhile, it had a negative correlation with weight loss, a value, total sugar, lycopene, and color index and TA which is indicated to fruit ripening. Carotenoids significantly and positively correlated with VC, L* value, appearance and negatively with C. index, a* value, lycopene content of tomato fruits.

In Figure 8 (heatmap), it can be clearly seen the changes in physicochemical properties of treated tomato fruits during storage periods. Generally, general appearance, pH, vitamin C, firmness, TSS, carotenoids, L value and b value of tomato fruits were gradually

reduced with the storage period; fruit weight loss, sugar content, color index, and lycopene were slowly increased. At the end of storage periods (28 + 2 d), highest content of vitamin C, firmness, TSS, carotenoids, L* value and b* of tomato fruits treated with chitosan and CaCl₂. Furthermore, these treatments also reduced the content of lycopene, value a, and color index than other treatments. This finding indicated that the treatment with chitosan and CaCl₂ were effective to delay the color changes, retard the ripening process and senescence of tomato fruit (Figure 6). Moreover, both treatments also maintained the well-appearance and alleviated decline the nutrient contents of tomatoes, such as vitamin C, total sugar, and total carotenoids.

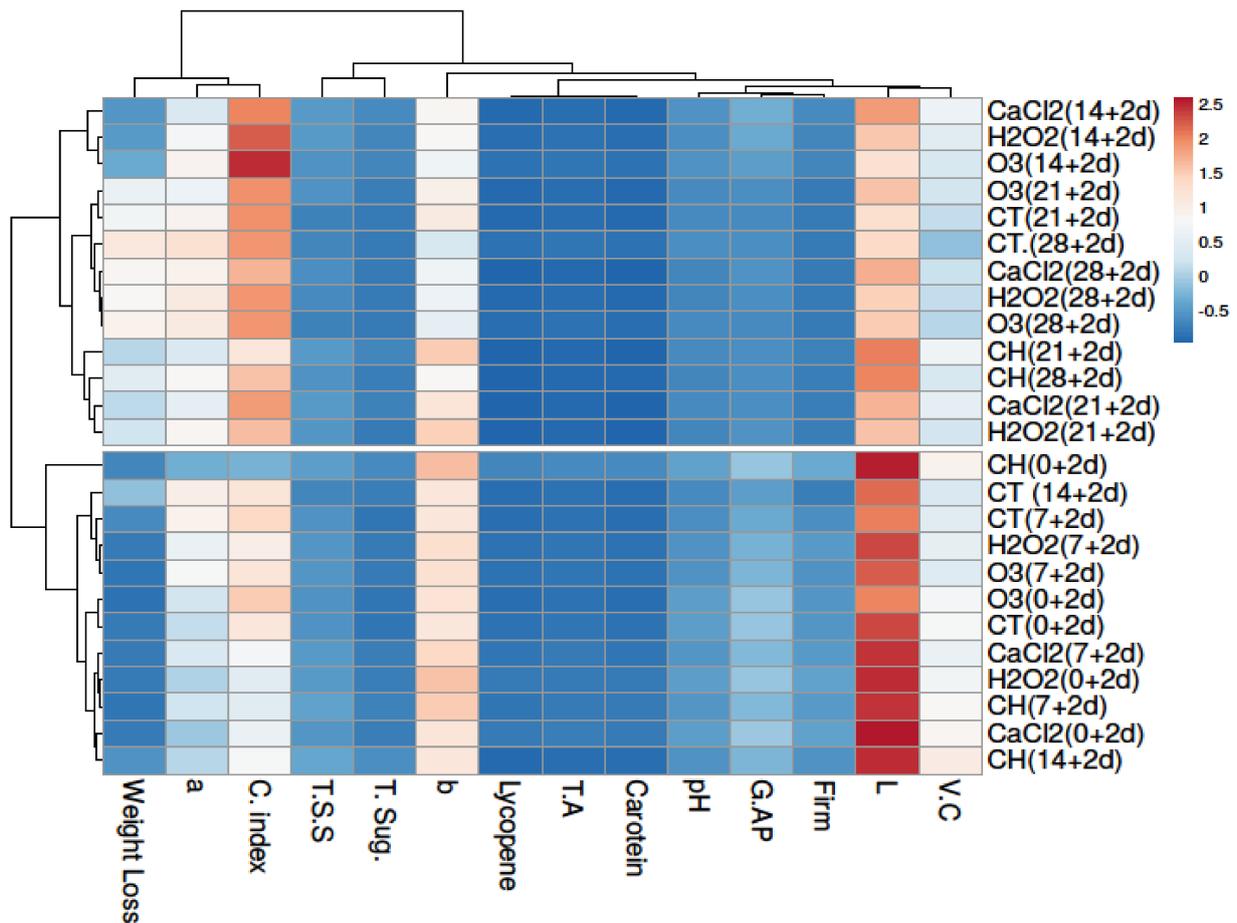


Figure 8. Correlation heat map between physiochemical quality of tomato fruit after 28 days of storage at 10 °C and following 2 days of storage at 20 °C. Positive relationships are presented in red color and negative relationships in blue color.

5. Conclusions

The results of this study indicate that tomato fruit treated with chitosan or CaCl₂ were the most applicable treatments for retarding tomato fruit ripening and extending shelf-life. Both treatments have a positive and favorable effect in preserving the physiochemical properties of tomato fruit during preservation. Treatments with chitosan or CaCl₂ reduced storage-induced alterations such as weight loss, TSS, firmness, pH, CI, and total lycopene and carotenoids while preserving the contents of sugars. The multivariate statistical analyses showed that the firmness and content of L*, a*, b*, TA, pH, carotenoids, lycopene, sugar, and TSS correlated with CI and general appearance. It can be concluded that these physiochemical qualities can be used as indicators for CI and appearance based on multivariate statistical analyses. However, treated tomato fruit had lower CI and higher appearance and nutrient value, particularly in those treated with chitosan and CaCl₂.

solutions. Finally, it implied that both previous treatments might be effective in regulating the ripening and delaying senescence of post-harvest fruit under cold storage conditions.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/horticulturae7090309/s1>, Table S1: Effect of treatments, storage period, and their interactions on physical and chemical parameters.

Author Contributions: Conceptualization, S.A.S. and K.F.A.; methodology, E.A.A. and M.M.A.M.; software, M.M.E.-M. and S.Z.A.; validation, K.F.A.; formal analysis, E.A.A.; investigation, M.M.E.-M.; resources, S.A.S.; data curation, M.M.A.M.; writing—original draft preparation, S.Z.A. and M.M.E.-M.; writing—review and editing, K.F.A., M.M.E.-M. and E.A.A.; visualization, S.A.S. and E.A.A.; supervision, S.A.S. and K.F.A.; project administration, M.M.A.M.; funding acquisition, S.Z.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Cairo University, Faculty of Agriculture, Giza, Egypt.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We express our thanks to Said Abdullah Shehata, Cairo University, Faculty of Agriculture, Giza, Egypt, for providing facilities via his running project “Increase export competition for vegetable crops”. Moreover, we thank Rwotonen I. Bob (Faculty of Agriculture, Makerere University) for contributing to the completion of this article.

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

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