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Research Article

The effect of *Aloe vera* and hot water treatments on maintaining the quality of guava fruit in cold storage

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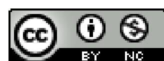
ABSTRACT- The guava fruit has a relatively short storage life due to its physicochemical properties. Despite many studies to maintain the quality and increase the storage life of guava fruits, no optimal method is currently available in this regard. To reduce the chilling injury of guava fruits, this study focused on the postharvest application of *Aloe vera* gel and hot water treatments on guava fruits based on a factorial experiment in a completely randomized design. The applied treatments were storage times at five levels (0, 7, 14, 21, and 28 days), hot water treatments at three levels (no hot water, 40, and 50 °C,) for 10 min, *A. vera* gel at three levels (0, 5, and 10%), and a control treatment (distilled water, *A. vera*-free + no hot water). Samples were stored at 7±1 °C with 95% relative humidity for 28 days and then at 24 °C for 24 h for adaptation to market conditions. According to the results, the values of the a* color index increased but the values of the L* and b* color indices decreased over storage time. Among the experimental treatments, the 5% *A. vera* gel was more effective for maintaining guava fruit quality by increasing the total sugar content and lowering the ion leakage percentage. The interaction of 40 °C hot water + 10% *A. vera* gel led to positive effects by reducing the respiration rate and better-preserving fruit firmness. The total chlorophyll values of all treatments in the 4th week were the lowest compared to the same treatments in weeks 1-3. An increase in the storage period resulted in a downward trend in ascorbic acid index. The minimum peel carotenoid and flavonoid content were observed in 40 °C hot water + 10% *A. vera* gel treatment. Treatments with no hot water + 5% *A. vera* gel, no hot water + 10% *A. vera* gel, and 40 °C water + 5% *A. vera* gel resulted in the highest levels of peel antioxidants, ion leakage, and malondialdehyde, respectively. The results of this study demonstrated that the use of *A. vera* gel and hot water treatments reduces the chilling injury of guava fruits, leading to a better storage life of fruits during the storage time.

INTRODUCTION

The use of fresh fruits and vegetables has been increasing with changes in lifestyle in the last decade (Mohammadi and Saidi, 2020). Horticultural crops are exposed to mechanical damage, temperature and moisture fluctuations, and contamination during transportation, ultimately resulting in postharvest decay. These crops are also susceptible to various postharvest diseases because of high moisture content, high levels of sugars and nutrients, and thin peels (Antunes et al., 2014). About 25% and 50% of horticultural crops are generally destroyed in industrial and developing countries, respectively, because of fungal diseases during storage and transportation. A variety of postharvest technologies have so far been employed to control decays caused by such diseases (Singh et al., 2010). The use of chemicals as pesticides is prohibited in many countries as these substances kill useful organisms and remain in the soil in toxic forms (Marín et al., 2017). On the other hand, there is increasing evidence that consumers tend to use organic agricultural products (Tadeo, 2008). Therefore, there is a need to pay attention to modern environmentally friendly approaches for the storage of agricultural products to provide human health as suggested by Reganold and Wachter (2016).

Guava, *Psidium guajava* L., is a fruit tree that plays an important role in the domestic economies of many tropical countries (Vitti et al., 2020). It belongs to the Myrtaceae family and the genus *Psidium.* It is native to southern and central America. This genus includes 150 species that are mostly edible and cultivated on a commercial scale (Gill et al., 2016). As the most popular and important species in this genus, the guava fruit is botanically a multi-grain kernel with a diameter of 2.5-10 cm and a size of 100-250 g. The fruit has a bright green peel, which turns yellow when it is ripe, but the fruit pulp may be pink, creamy, white, or yellow, and the fruit texture may be smooth or coarse. As a fleshy fruit with various thicknesses, guava has a sweet strong smell and typically contains hard, yellowish, and unpalatable seeds (Dube and Singh, 2019).

Guava originated from an area extending from southern Mexico through Central America (Laily et al., 2015). The year of guava′s arrival in Iran is unknown, but Bandar Abbas, Minab, Roudan, Siahoo, Chabahar, Sarbaz, and Nikshahr in Iran are the cities where guava is produced



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in them. In addition to domestic use, the fruit is exported to countries along the Persian Gulf (Etemadipoor et al., 2019).

Guava fruit ripening is associated with considerable changes in the biochemical composition of fruits. The concentrations of soluble solids and total sugars increase during ripening. The fruit sugar profile differs in various varieties, but fructose is the dominant sugar in ripened guava fruits, followed by glucose and sucrose (Arévalo-Marín et al., 2021). The contents of titratable acidity and total phenols generally decrease during fruit ripening. The main organic acid is citric acid, followed by malic and glycolic acids. The content of ascorbic acid rises dramatically during early fruit ripening stages and then declines with aging. The strong smell of guava fruits results from the presence of esters and terpenes, and volatile aromatic compounds increase when the fruit ripens (Sridevi, et al., 2018).

Since fruit growth and crop postharvest conditions in tropical regions provide an appropriate milieu for the proliferation of primary and secondary pathogens, fruit decay is very widespread in these regions and is mostly controlled by chemical treatments (Pétriacq et al. 2018). The guava fruit quality is affected by pre-harvest factors, such as fruiting season, gardening practice, canopy situation, tree age, and the use of micronutrients and growth regulators at the pre-harvest stage. Postharvest factors, including temperature, physical damage, dryness of the fruit, chilling injury, physiological disorders, and pathobiological abnormalities, also influence fruit quality. To prevent and reduce the adverse effects of these factors, it is recommended to use treatments such as cold and hot treatments, radiation, biological pest control, various edible coatings, and exposure to the modified atmosphere (Pétriacq et al. 2018).

Khaliq et al. (2019) investigated the effect of *Aloe vera* gel coating alone and in combination with *Fagonia indica* extract on the physiological and biochemical properties of sapodilla *(Manilkara zapota)* fruits. The addition of 1% *F. indica* extract to 50% or 100% *A. vera* gel resulted in significantly higher levels of ascorbic acid, flavonoids, total phenols, and inhibition of radicals. They concluded that adding *F. indica* extract to *A. vera* gel could prolong the storage period of sapodilla and preserve its quality during storage (Khaliq et al., 2019). Dutta et al. (2017) studied the effects of hot water, on storage life, postharvest disease spread, and changes in physicochemical properties during storage of guava. They reported that hot water treatment increased fruit resistance to diseases in winter (Dutta et al., 2017).

No study is available on the use of hot water treatment with *A. vera* gel edible coating to increase storage life and control chilling injury in guava fruits. Therefore, the present study aims to increase the storage life of guava fruits treated with *A. vera* gel as the edible coating with hot water treatment.

MATERIALS AND METHODS

Fruit Materials

Guava fruits were harvested from a commercial garden in Minab City and transferred to the agriculture laboratory at the Faculty of Agriculture and Natural Resources, Hormozgan University. Fruit samples were selected according to their appearance and the absence of diseases and peel damage. Fruits were then disinfected with 5% sodium hypochlorite for 1 min, washed with distilled water, and air-dried for 30 min. At this stage, the applied treatments were hot water immersion at three levels including no hot water treatment, and 40 °C, and 50 °C water treatments, *A. vera* gel inoculation were conducted at three levels including 0, 5, and 10%, and five storage times including 0, 7, 14, 21, and 28 days were used. *Aloe vera* coating was prepared according to Misir et al., 2014. A group with zero concentrations of treatments was considered the control. After applying the experimental treatments, samples were stored in a fridge at 7±1 °C with 95% relative humidity for 28 days and then at 24 °C for 24 h for adaptation to market conditions. The experiments were done periodically once a week.

Fruit Color and Firmness

The skin color of guava fruits was investigated by using a colorimeter. Three different areas of fruit were used for the measurement of L*, a*, and b* indices, and these values were used for calculating hue angle and chroma. Before using the colorimeter, the colorimeter was warmed and calibrated. The letters L*, a*, and b* represent each of the three values of the International Commission on Illumination LAB (CIELAB) color space used to measure objective color and calculate color differences. L* represents lightness from black to white on a scale of zero to 100, while a* and b* represent chromaticity with no specific numeric limits (Hristova et al. 2018).

Fruit firmness was measured by using a digital handheld fruit penetrometer with an 8 mm tip. Average fruit firmness was expressed as newton (N) and was measured using a durometer (Dutta et al., 2009).

Determination of Ascorbic Acid

Ascorbic acid was determined by titration method using 2,6-dichloro-indophenol as described in Official Methods of Analysis (AOAC, 1990).

Fruit Weight Loss

Fruit weight loss was calculated according to Samra et al., (2019). The difference between the final and initial weights (±0.01 g) of each replication on respective sampling days was used to assess fruit weight loss (%).

Respiration Index

Respiration of the fruits was determined using three fruits per treatment at regular intervals of 4 days during the storage period. The respiration was measured at 25 °C and the guava fruits were equilibrated at this temperature before the measurements were taken. Guava fruits with known weight were placed in a 1000 m airtight plastic container fitted with a rubber septum on the lid. A headspace analyzer 8 (Dansensor, Checkmate 9900, Denmark) consisting of a syringe was inserted into the container through the rubber septum, to measure the respiration rate.

Soluble Solid Content (SSC) and Acidity Measurement

To evaluate the chemical properties, the SSC of fruits was determined by a digital refractometer at room temperature based on the Brix degree and as a percentage. The fruit acidity was measured in the fruit extract by a pH meter, and the solution pH was read at 20 °C. The titratable acids (TA) were assessed by the 1% sodium hydroxide titration method and calculated as a percentage per gram of the fruit extract according to Khan et al. (2008). A total of 10 mL of extracted guava juice was diluted with 40 mL of distilled water and titrated against NaOH (0.1 N) until the solution attained pH 8.2.

Total Phenolic Content (TPC)

TPC was determined according to the modified method by Meyers et al. (2003). A reaction mixture was prepared by adding 300 µL of methanolic extract to a solution prepared from 1.2 mL of sodium carbonate (7%) and 1.5 mL of Folin-Ciocalteu reagent (10%). The final mixture was incubated in dark conditions at room temperature (25 °C) for 1.5 h. Thereafter, the absorbance was recorded at 765 nm wavelength using the UV/VIS-spectrophotometer. Gallic acid was used as standard, and the results were expressed as mg GAE g^{-1} fresh weight (FW).

Total Flavonoid Content (TFC)

TFC was estimated by using a colorimetric method (Meyers et al. (2003). A total of 500 µL of methanolic extract was added to 100 µL of aluminum chloride (10%) and 100 µL of acetate potassium (1 mM). The resulting mixture was incubated for 30 min and the absorbance was recorded at 415 nm wavelength using the UV/VIS-spectrophotometer. Quercetin was used as a standard, and the results were expressed as mg QE g^{-1} FW.

Malondialdehyde Content

The malondialdehyde (MDA) content was quantified using the method described by Pasquariello et al. (2015) with some modifications. A total of 1 g of fruit tissue was homogenized with 15 mL of trichloroacetic acid (10%; v/v) and the homogenate was centrifuged for 20 min at 10,000× *g*. A total of 2 mL of supernatant was collected and mixed with 2 mL of 2-thiobarbituric acid (0.6%, w/v). The reaction mixture was heated for 20 min at 100 °C in a water bath. Subsequently, after cooling, the absorbance of the reaction mixture was measured at 450 nm, 532 nm, and 600 nm wavelengths using the UV/VIS-spectrophotometer, and malondialdehyde content was determined as µmol kg^{-1} FW.

Determination of Antioxidant Activity

The antioxidant activity of the fruit extract against 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was determined by UV/Visible spectrophotometry at 517 nm wavelength.

Total Chlorophyll and Carotenoid Contents

Total chlorophyll and carotenoid contents were estimated according to the method used by Zhu et al. (2009) and Lichtenthaler (1987), respectively. A total of 1 g of persimmon tissue was homogenized with 5 mL of chilled acetone (80%) in dark and cool conditions. The homogenate was then centrifuged at 10,000× *g* for 5 min. The supernatant was collected, and reaming debris was re-extracted by adding 5 mL of acetone and repeating the above-mentioned process. Absorbance was noted at 470 nm, 646 nm, and 663 nm wavelengths using the UV/VIS-spectrophotometer after thoroughly mixing both supernatants. Total carotenoids were expressed as µg g^{-1} FW.

Data Analysis

This research was conducted as a factorial experiment in a completely randomized design with three replications. The normality of obtained data and homogeneity of variances was tested using Kolmogorov–Smirnov and Levene's tests, respectively. The cultivars were compared by the analysis of variance (ANOVA) and Duncan's test at a probability level of $P \leq 0.01$. Correlation coefficients were obtained using the Pearson test. Graphs were drawn by Excel software, in which columns represent the average of three replications and vertical bars indicate standard errors (± SE).

RESULTS

According to the results of ANOVA for the color indices, the L*, a*, and b* indices were significantly affected by the storage time and treatments, as well as the interaction of them. However, increasing the storage time led to reductions in L* and b* indices and an increase in a* index. For example, the highest reduction (21.87 units or 62.74%) in the L color index occurred in T4 treatment in the 4th week compared to the control treatment T1 (Fig. 1). However, the value of T4 treatment in the 4th week did not differ with the values of all other treatments except the value of T5 treatment in the same week. The value of T1 treatment in the 1st week did not significantly differ with the values of T2 and T3 treatments in the same week. The least reduction (22.27 units or 12.59%) was recorded in no hot water + 5% *A. vera* (T4) treatment compared to the control (T1) treatment in the 4th week (Fig. 1).

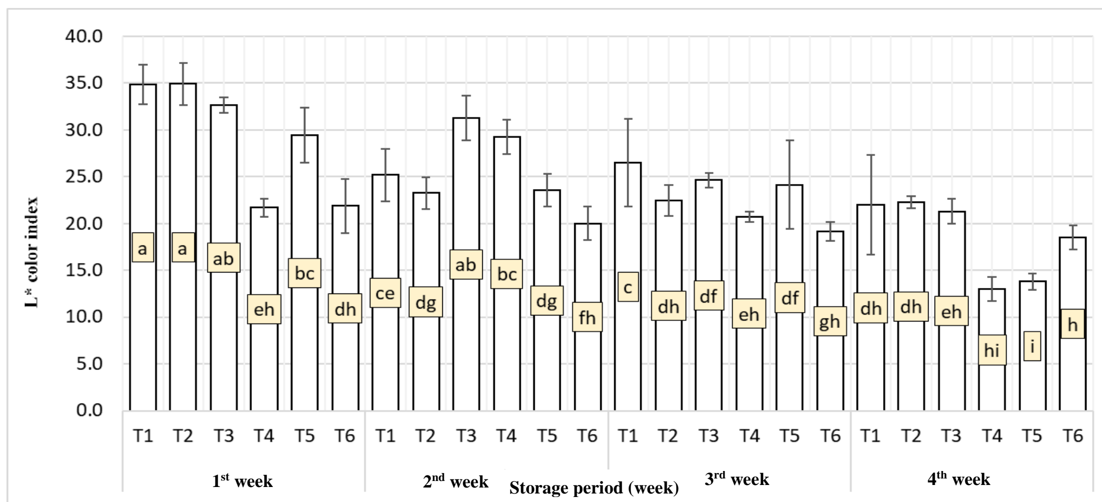

Fig. 1. Effect of hot water, and *A. vera* treatments on the L* color index. The vertical and horizontal axes represent the L* color index (units) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (Duncan's test at a probability level of $P \leq 0.01$).

The ANOVA test indicated that the fruit firmness index was significantly influenced by the simple and interaction effects of both time and treatment at the 0.01 significance level. An increase in the firmness index was observed with increasing the storage period. Among the different treatments, the highest increase (25.59 Newton or 178.77%) belonged to 40 °C water + 10% *A. vera* (T6) treatment compared to the control treatment (T1) in the 4th week (Fig. 2).

Based on the results of ANOVA, the simple and interaction effects of both time and treatment significantly influenced the weight loss index at a significance level of 0.01. This index increased significantly with increasing the storage period, and the highest increase (24.60 units) was recorded in 40 °C water + 10% *A. vera* (T6) treatment in the 4th week (Fig. 3).

The examination of ANOVA results revealed that the simple and interaction effects of both time and treatment significantly affected the total chlorophyll index at a significance level of 0.01. Increasing the storage period reduced this index significantly, and the highest reduction was measured in 40 °C hot water + 5 % *A. vera* (T5) treatment in the 4th week. However, the value of this treatment did not significantly differ from the values of the control treatment (T1) and other treatments in the same week (Fig. 4).

The carotenoid index was significantly influenced by the simple and interaction effects of both time and treatment at the 0.01 significance level. A reduction in the carotenoid index was noticed with increasing the storage period. The greatest reduction belonged to T5 and T6 treatments in the 4th week. However, the value of each of those treatments did not significantly differ from the values of the control treatment (T1) and other treatments in the same week (Fig. 5).

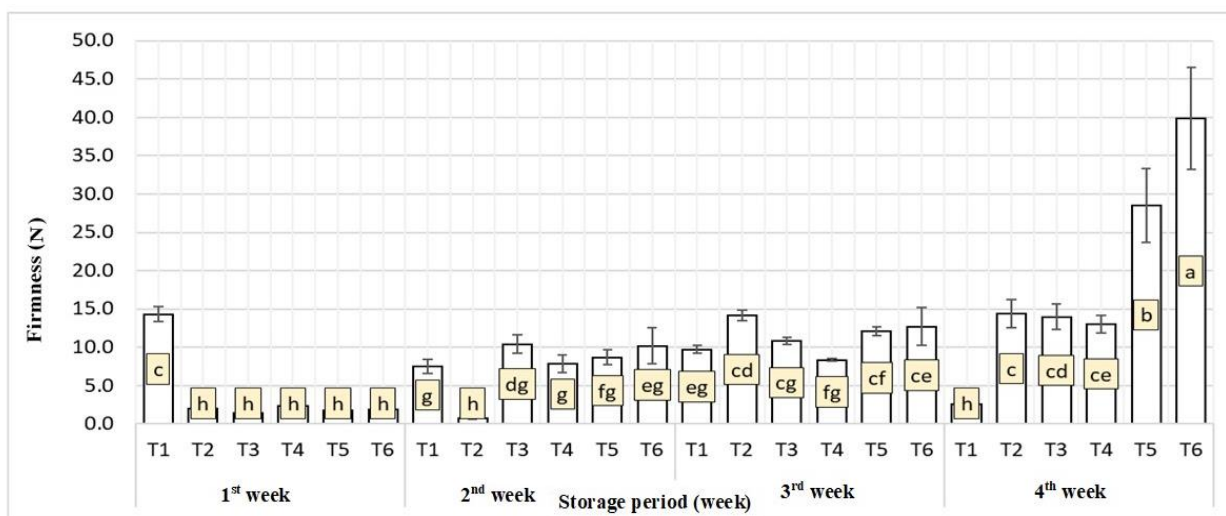

Fig. 2. Effect of hot water, and *A. vera* treatments on the firmness index. The vertical and horizontal axes show the firmness index (Newton, N) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

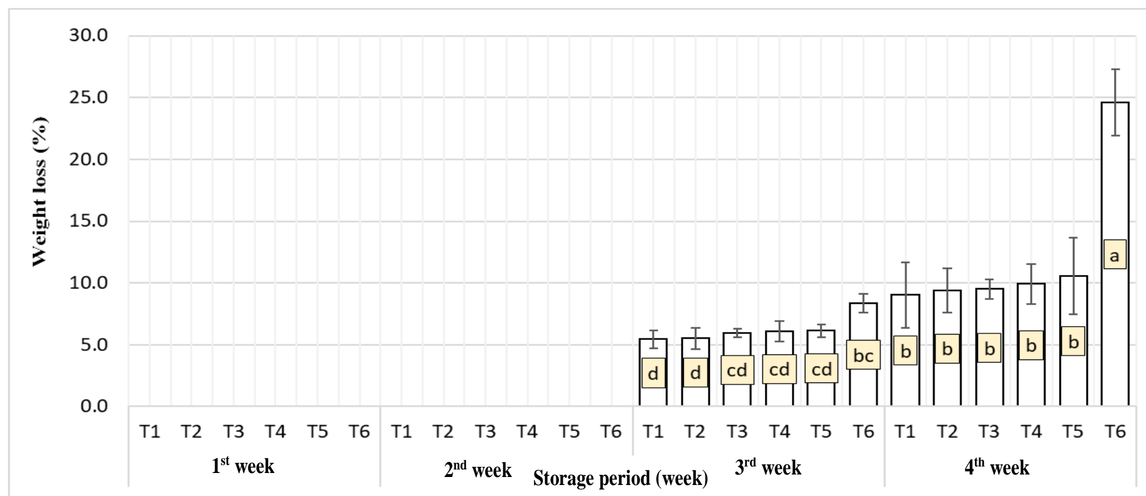

Fig. 3. Effect of hot water, and *A. vera* treatments on the weight loss index. The vertical and horizontal axes display the weight loss index (%) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

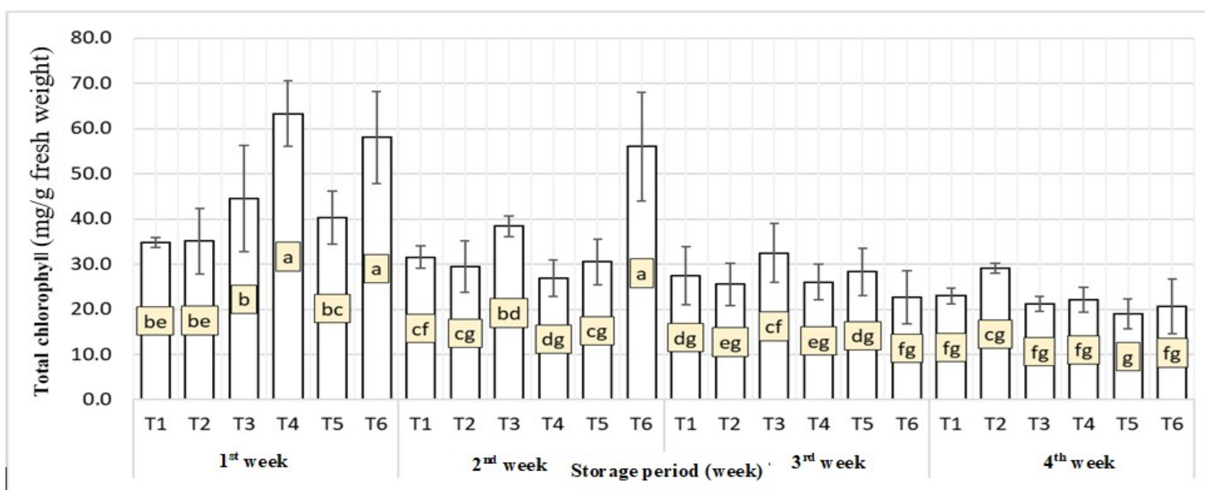

Fig. 4. Effect of hot water, and *A. vera* treatments on the total chlorophyll index. The vertical and horizontal axes indicate the total chlorophyll index (mg/g fresh weight) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (Duncan's test at a probability level of $P \leq 0.01$).

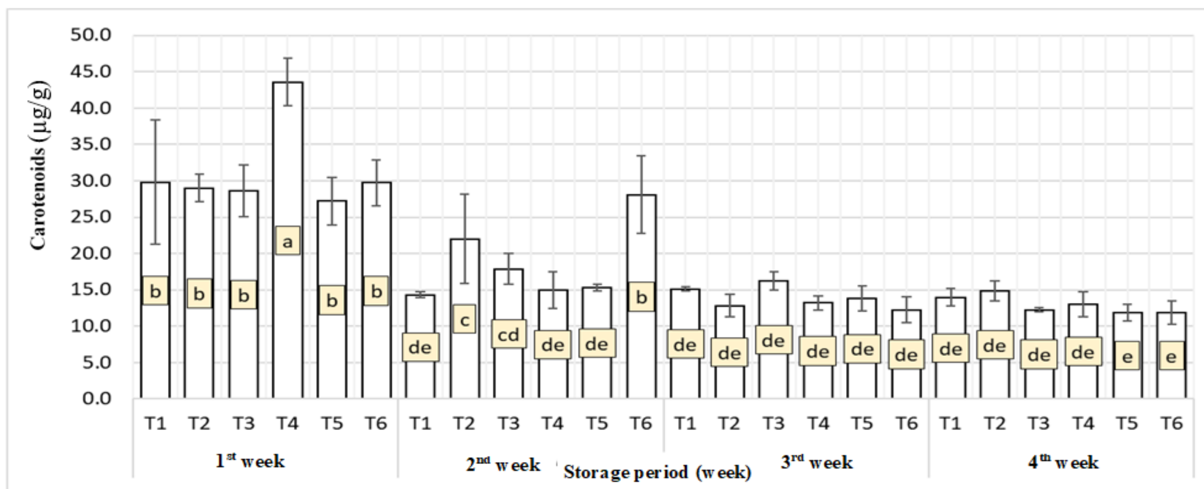

Fig. 5. Effect of hot water, and *A. vera* treatments on the carotenoid index. The vertical and horizontal axes indicate the carotenoid index (μg/g) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

The peel antioxidant index was significantly affected by the simple and interaction effects of time and treatment at a significance level of 0.01. An increase occurred in this index with an increase in the storage period. Among the experimental treatments, the 5% *A. vera* + no hot water (T2) treatment presented the uppermost rate (88.98 units or 915.83%) of this index in comparison to the control treatment (T1, *A. vera*-free + no hot water) in the 3rd week (Fig. 6). However, the value of T2 treatment in this index did not have a significant difference at the 1% level with the value of T3 treatment in the 3rd week (Fig. 6).

In addition, increasing the storage period led to a growing trend in the antioxidant index of the fruit pulp, and the utmost rise (77.13 units or 702.41%) of this index was measured in the 3rd-week (Fig. 7).

The comparison of obtained data by the ANOVA indicated that both the simple and interaction effects of time and treatment significantly affected the ascorbic acid index at the 0.01 level. An increase in the storage period resulted in a downward trend in this index. The greatest value of this index belonged to T6 treatments in the 1st and 2nd weeks and the lowest value belonged to T1-T5 treatments in the 4th week (Fig. 8).

The results of the current study showed an increasing trend in the ion leakage index with increasing the storage period. Among the different treatments, the highest increase (24.85 units or 21.03%) of this index was recorded in the 10% *A. vera* + no hot water (T3) treatment in the 4th week (Fig. 9). However, the value of T3 treatment of this index in the 4th week did not have a significant ($P \leq 0.01$) difference with the value of T2 treatment of the same index in the 3rd week (Fig. 9).

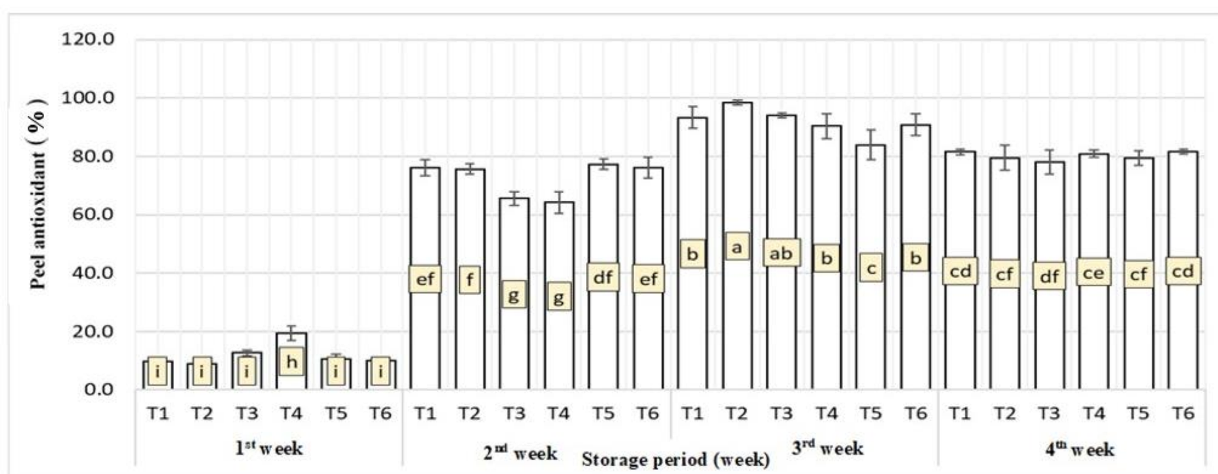

Fig. 6. The two-factor mixed effects of time, hot water, and *A. vera* treatments on the peel antioxidant index. The vertical and horizontal axes show the peel antioxidant index (%) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

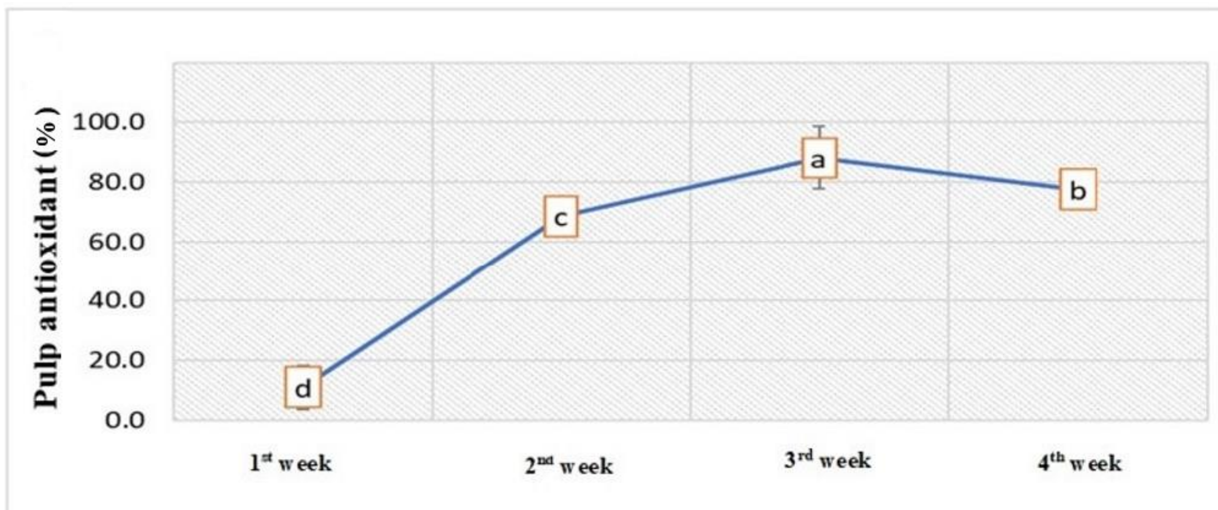

Fig. 7. The simple effect of time treatment on the fruit pulp antioxidant index. The vertical and horizontal axes represent the pulp antioxidant index (%) and the storage period (weeks), respectively. The values of treatments specified with different letters are significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

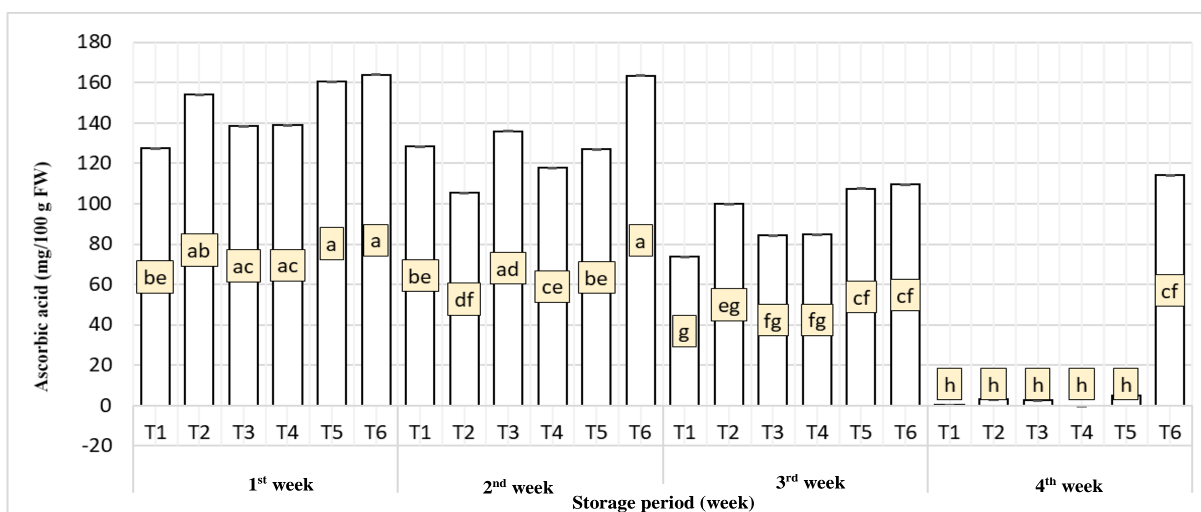

Fig. 8. The two-factor mixed effects of time, hot water, and *A. vera* treatments on the ascorbic acid index. The vertical and horizontal axes display the ascorbic acid index (mg/100 g FW) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

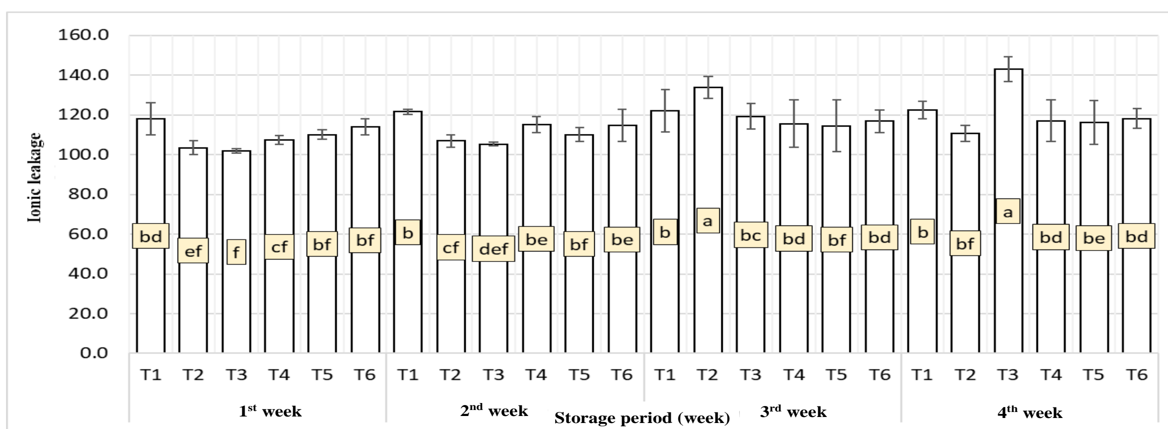

Fig. 9. Effect of hot water, and *A. vera* treatments on the ion leakage index in guava fruit. The vertical and horizontal axes display ionic leakage index and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

Both the simple and interaction effects of time and treatment significantly affected the MDA index at a significance level of 0.01. This index had a rising trend and increased with the passage of time from the 3rd week to the 4th week. Among the different treatments, the 40 °C water + 5% *A. vera* (T5) treatment showed the greatest rate (39.55 units or 736.56%) of this index compared to the control treatment (T1, *A. vera*-free + no hot water) and other treatments in the 4th week (Fig. 10).

Pulp phenol decreased with an increase in the storage period time from the 3rd week to the 4th week. Among the different treatments, the highest (1366 units or 89.56%) and the lowest (388 units or 25.41%) reductions of this index were respectively noticed in the no hot °C water + 0% *A. vera* treatment (control treatment, T1) and 40 °C water + 10% *A. vera* treatments (T6) both in the 4th week (Fig. 11). However, the value of T1 treatment in the 4th week did not significantly differ with the values of all other treatments except the value of T6 treatment in the same week (Fig. 11).

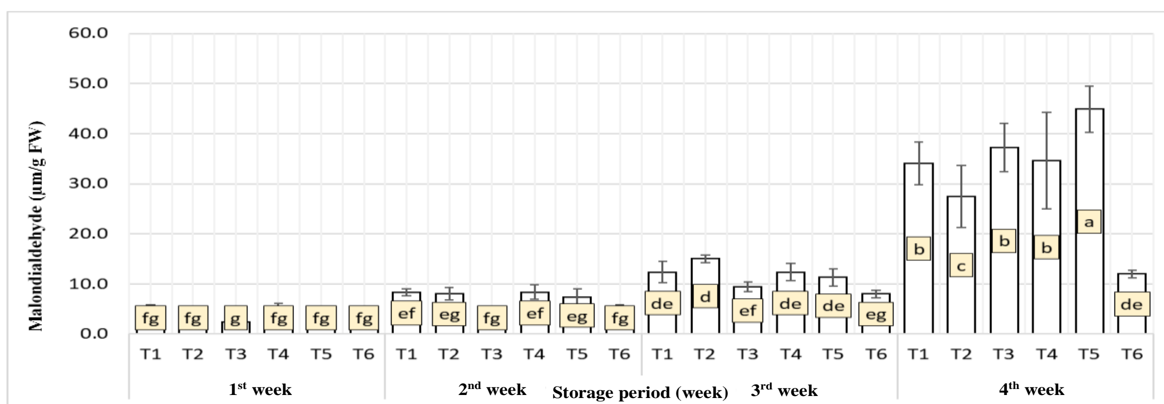

Fig. 10. Hot water, and *A. vera* treatments on the MDA index. The vertical and horizontal axes show the MDA index (µm/g fruit FW) and the storage period (weeks), respectively. respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

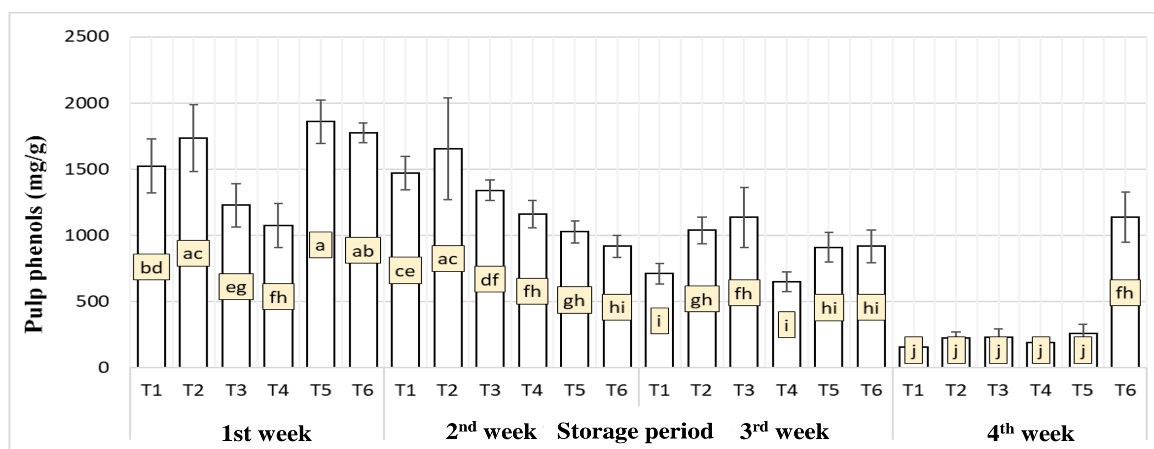

Fig. 11. The two-factor mixed effects of time, hot water, and *A. vera* treatments on the pulp phenol index. The vertical and horizontal axes represent the pulp phenol index (mg/g) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

Based on the results obtained by the ANOVA test, both the simple and interaction effects of time and treatment significantly influenced the peel phenol index at a significance level of 0.01. An increase in the storage period reduced this index, with the highest (1134 units or 85.95%) reduction recorded in 10% *A. vera* + no hot water (T3) treatment in the 4th week (Fig. 12). However, the value of T3 treatment of this index in the 4th week did not significantly differ with the values of all other treatments except the value of T6 treatment in the same week (Fig. 12).

The calculations of the ANOVA test indicated that the peel flavonoid index was significantly affected by both the simple and interaction effects of time and treatment at the 0.01 significance level. A downward trend occurred in this index with increasing the storage period from the first week onwards, with the most (9774 units or 81.35%) decrease measured in 40 °C water + 10% *A. vera* treatment (T6) in the 3rd week (Fig. 13). However, the value of T6 treatment in the 3rd week did not significantly differ with the values of the most other treatments in the 2nd, 3rd and 4th weeks (Fig. 13).

According to the examination of obtained data by the ANOVA test, both the simple and interaction effects of time and treatment significantly affected the pulp flavonoid index at a significance level of 0.01. This index showed a descending trend over time with increasing the storage period from the first week onwards, and the *A. vera* free + no hot water treatment (T1) presented the utmost (1366 units or 89.56%) decline in the 4th week (Fig. 14). However, the value of T1 treatment in the 4th week did not significantly differ with the values of T4 and T6 treatments in the same week (Fig. 14).

The analysis of obtained data by the ANOVA test indicated that the soluble solid index was significantly influenced by both the simple and interaction effects of time and treatment at the 0.01 significance level. Increasing the storage period led to an increase in this index. However, the value of this index decreased in all the treatments of the second week except the T1 treatment compared to the value of corresponding treatments in the first week. That is, with the increase in the storage period, from the first week to the second week, there was a decreasing trend, but from the second week onwards, there was an increasing trend in the value of this index. The reason for this was not clear. Anyway, the 40 °C water + 5% *A. vera* (t5) treatment showed the greatest rate of this index (1.94 units or 36.88%) in the 4th week (Fig. 15). However, the value of T5 treatment of this index in the 4th week did not significantly differ with the values of the most other treatments in the 1st, 3rd and 4th weeks (Fig. 15).

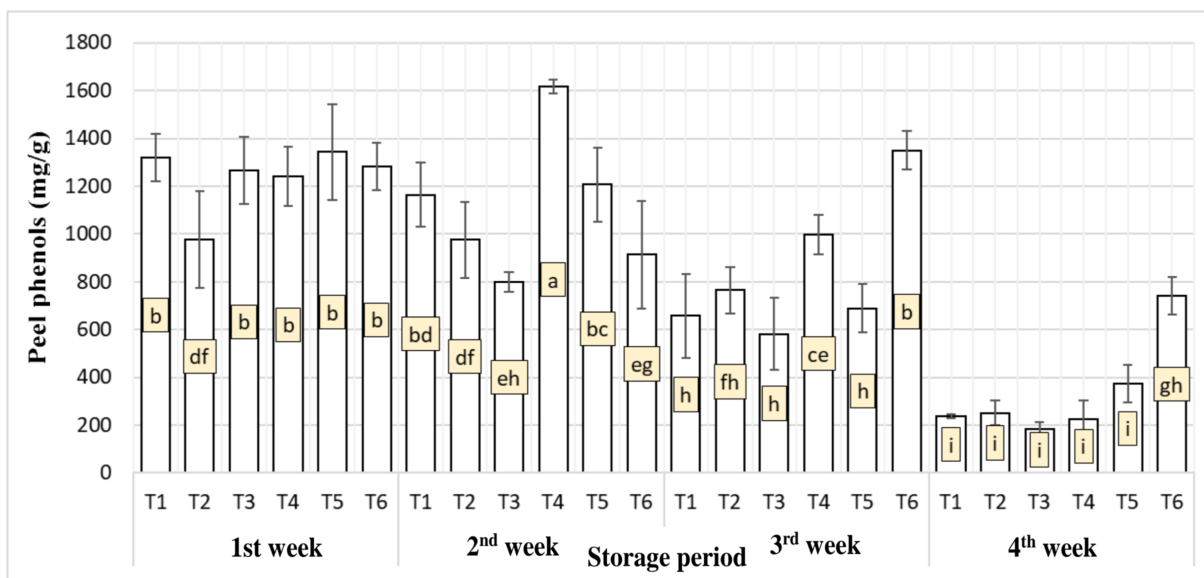

Fig. 12. The two-factor interaction effects of time, hot water, and *A. vera* treatments on the peel phenol index. The vertical and horizontal axes represent the peel phenol index (mg/g) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

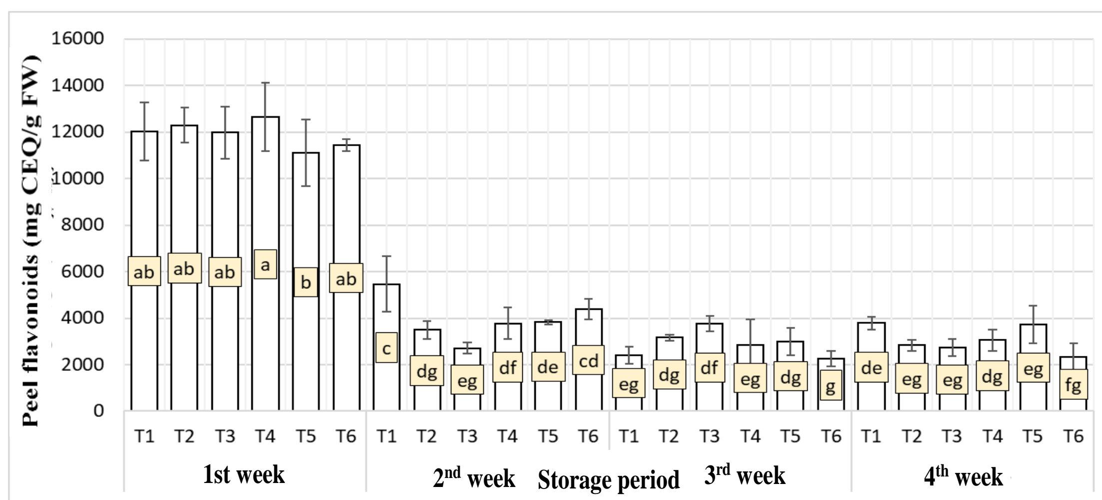

Fig. 13. The two-factor mixed effects of time, hot water, and *A. vera* treatments on the peel flavonoid index. The vertical and horizontal axes represent the peel flavonoid index (mg CEQ/g FW) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

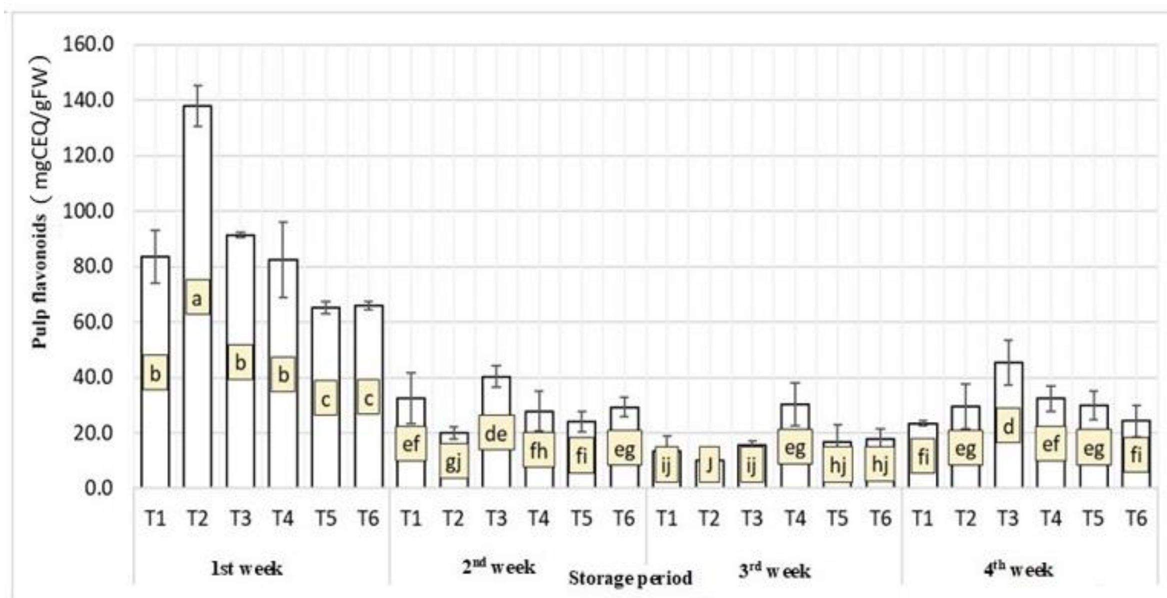

Fig. 14. The two-factor mixed effects of time, hot water, and *A. vera* treatments on the pulp flavonoid index. The vertical and horizontal axes represent the pulp flavonoid index (mg CEQ/g FW) and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

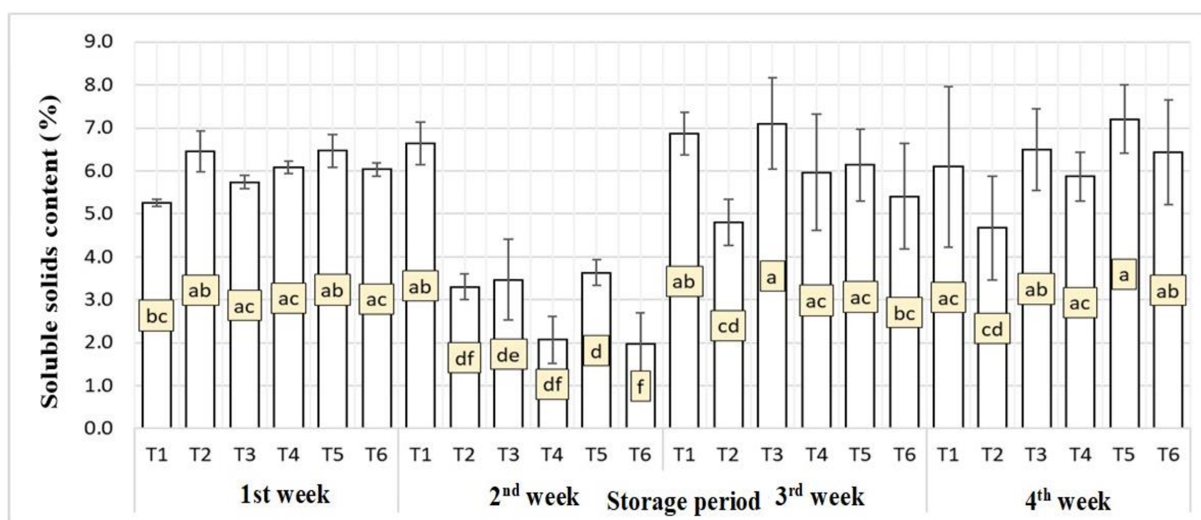

Fig. 15. The two-factor mixed effects of time, hot water, and *A. vera* treatments on the soluble solid index. The vertical and horizontal axes show the soluble solid index based on the Brix unit and the storage period (weeks), respectively. T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

The results of ANOVA showed that the pH index was significantly affected by the simple and interaction effects of time (Fig. 16) and treatment (Fig. 17) both at a significance level of 0.01. An increase in the storage period elevated this index, with the greatest increase (0.57 units or 13.96%) recorded in the 4th-week. However, there was not a significant difference ($P \leq 0.01$) between the values of this index in the 3rd and 4th weeks of storage (Fig. 16).

Based on the ANOVA table, the simple and interaction effects of time and treatment significantly influenced the TA index both at the 0.01 significance level. The highest rate (0.34 units or 50.75%) of this index was measured in the 2nd-week treatment (Fig. 18).

The examination of the ANOVA table demonstrated that the respiration index was significantly influenced by the simple and interaction effects of time and treatment factors both at a significance level of 0.01. With the increase of the storage period, the value of this index did not show a regular increasing trend. However, the uppermost (0.37%) increase of this index was observed in the 40 °C water + 5% *A. vera* (T5) treatment in the 3rd week (Fig. 19).

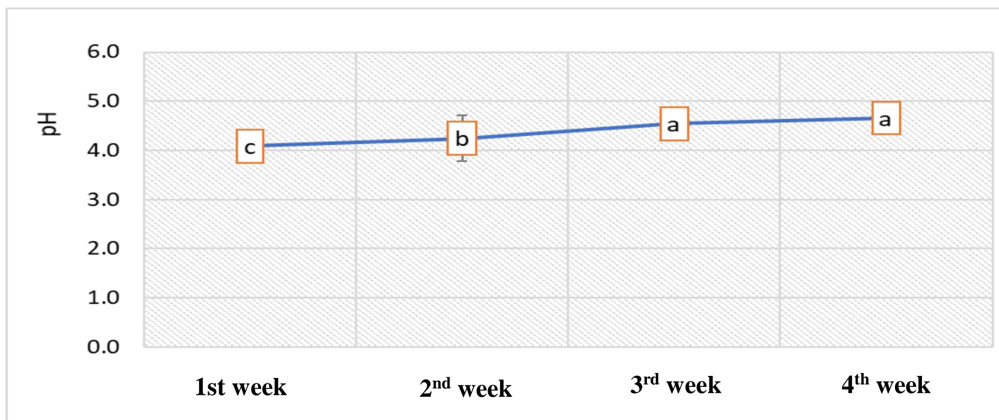

Fig. 16. The simple effect of storage time on the pH index. The vertical and horizontal axes display the pH index and the storage period (weeks), respectively. The values of treatments specified with different letters are significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

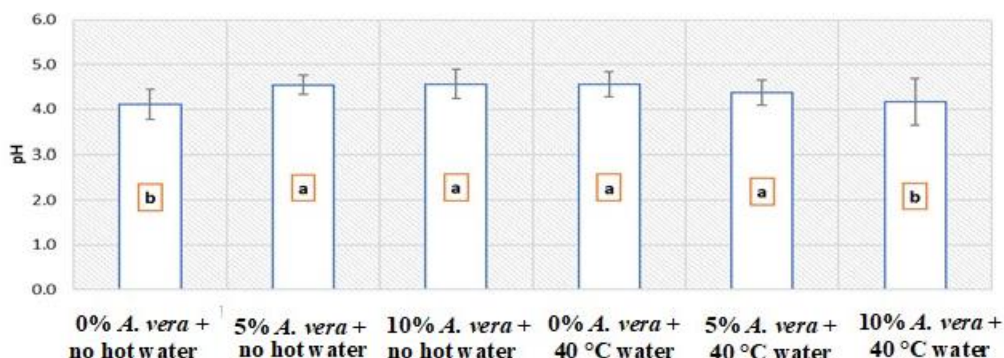

Fig. 17. The simple effect of hot water and *A. vera* treatment on the pH index. The vertical and horizontal axes show the pH index and the *Aloe vera* gel and hot water treatments in the 4th week, respectively. The values of treatments specified with different letters are significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

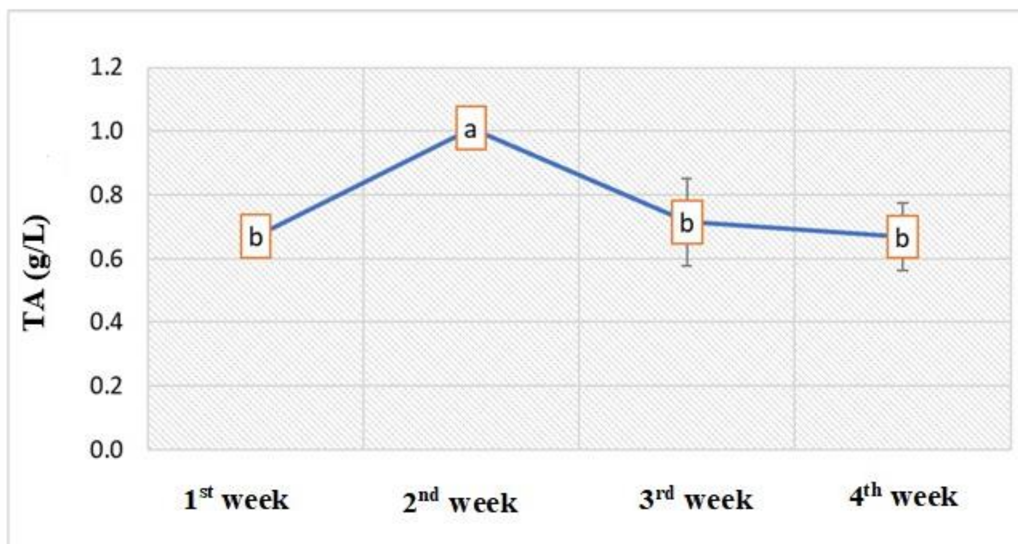

Fig. 18. The simple effect of the time treatment on the TA index. The vertical and horizontal axes represent the TA index (g/L) and the storage period (weeks), respectively. The values of treatments specified with different letters are significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

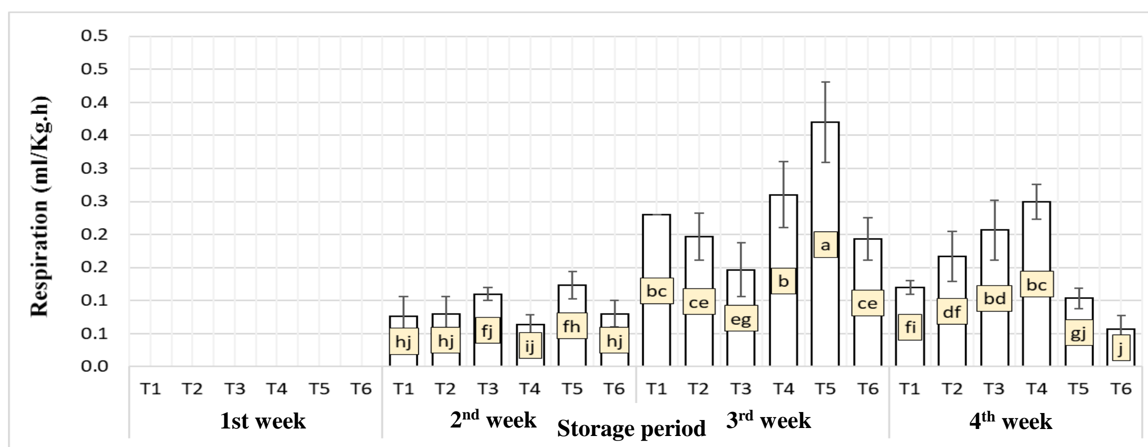

Fig. 19. The two-factor mixed effects of time, hot water, and *A. vera* treatments on 2. The vertical and horizontal axes show the respiration index (ml/Kg.h) and the storage period (weeks), respectively T1: *A. vera*-free + no hot water; T2: 5% *A. vera* + no hot water; T3: 10% *A. vera* + no hot water; T4: *A. vera*-free + 40 °C hot water; T5: 5% *A. vera* + 40 °C hot water and T6: 10% *A. vera* + 40 °C hot water. Treatments sharing the same letter on the related columns are not significantly different (ANOVA test and Duncan's test at a probability level of $P \leq 0.01$).

DISCUSSION

Fruit color is an important quality parameter evaluated by consumers and can also represent crop quality. The color and lightness of the fruit peel are generally the most important fruit quality indices. The results of this study indicated that increasing the storage period reduced the L* color index. Evidence indicates that the L* level decreased with fruit browning over time caused by the growth of yeasts and bacteria, oxidation reaction, and enzymatic activities (Pasquariello et al. 2015). On the other hand, the a* color index increased with increasing the storage period. An increase in the a* color level during fruit storage has been shown to result from ripening, aging, or advanced non-enzymatic browning of the fruits (Etemadipoor et al., 2019). A reduction occurred in the b* color index with increasing the storage period. Both enzymatic and non-enzymatic reactions have been reported to cause changes in fruits (Tian et al. 2006). The darkening of fruits is probably caused by the release of phenolic compounds from vacuoles and their oxidation by the polyphenol oxidase enzyme (Zhu et al., 2009).

In this study, the total chlorophyll content decreased with the increased storage period. The reduced respiration and delayed aging by the applied treatments could prevent chlorophyll degradation in fruits and maintain their green color and marketability during the storage period, which corresponds to the report of Bagnazari et al. (2018). Increasing the storage period also led to a decrease in the carotenoid index. Carotenoids are the widest group of pigments in nature that create yellow to red colors in fruits. Fruit ripening includes a series of complex biochemical reactions that lead to the production of carotenoids and aromatic substances (Hosseinpour et al., 2017). The fruit carotenoid content is different depending on the maturity degree of fruits, and the carotenoid content is affected by the geographical region, as well as differences between cultivars. In a similar experiment, hot water treatment reduced carotenoid content and delayed chlorosis in tomatoes (Khaleghi et al., 2011).

According to the findings of this study, the increased duration of guava fruit storage resulted in elevated levels of peel and pulp antioxidant indices of this fruit. It has been shown that vitamin C (ascorbic acid) and phenolic compounds account for the most important fruit antioxidant compounds and their preservation during the postharvest period elevates the antioxidant capacity of fruits and preserves their quality (Bagnazari et al., 2018). As stated by Dragovicuzelac et al., (2007), a decrease in gas exchange, including reduced oxygen entry to the fruit, creates a covering layer by the fruit covers, thereby reducing the oxidation of acids, phenols, and other compounds, such as ascorbic acid The preservation of these antioxidant compounds may be a reason for maintaining the total antioxidant capacity of fruits in the treatments compared to control samples during storage (Sudarshan et al., 1992). The results presented in the current study revealed a reduction in the ascorbic acid index with increasing the fruit storage period. Vitamin C declined in most fruits during storage, which was assumed to be caused by ascorbic acid instability and its sensitivity to oxygen, light, temperature, and biotic and environmental stresses. The use of ascorbic acid, as an electron donor to neutralize free radicals, has also been confirmed, which is another reason for the reduction of ascorbic acid as an important antioxidant over time (Sudarshan et al., 1992). It has been reported that increasing oxidative metabolism increases reactive oxygen species (ROS), which can destroy biological membranes (Ismail et al., 2010). To prevent ROS damage, plants activate their antioxidant systems, including enzymatic systems such as ascorbate peroxidase, or non-enzymatic systems such as ascorbic acid (Sudarshan et al., 1992). An investigation on fresh apricots disclosed that a methyl cellulose-based edible coating caused water loss, and in combination with ascorbic acid, reduced vitamin C loss. Accordingly, the treatment applied in this research apparently reduced ascorbic acid. The results of the current study are also in line with a study on guava by Ismail et al. (2010).

The findings presented in this study indicated that increasing the storage period resulted in an increase in

the ion leakage index. The main reasons for the reduced ion leakage in the fruits compared to control samples include the prevention of weight loss, delayed aging, the reduction of stress on fruits by preserving antioxidant compounds, and decreased respiration (Mohammadi and Saidi, 2020). Similarly, Singh et al. (2010) found that the use of coating treatments reduced stress during storage, which resulted in decreased ion leakage, and more preservation of catalase and other antioxidant compounds, including vitamin C and total phenol, thereby increasing the antioxidant capacity compared to the control. Likewise, Mohammadi and Saidi (2020) reported a reduction in the ion leakage of bell peppers using chitosan edible coating.

In this research, the MDA index rose with the rise of storage time. MDA is considered the product of lipid peroxidation plant cell membrane (Liu et al., 2018). Lipid oxidation is generally an important indicator of damage to the membrane system and exacerbation of cell metabolism (Liu et al., 2018). MDA accumulation which increases the accumulation of brown polymers, leading to pericarp browning, can destroy the cell membrane composition (Kabasakalis et al., 2000). Similar to the observations of this study, Liu et al. (2018) observed that the MDA content was significantly lower in melatonin-treated strawberries than in control fruits. Phenolic compounds are one of the defense mechanisms of cells against adverse factors that decrease gradually with aging (Asghari and Khalili, 2018). In this study, reductions in both pulp and peel phenol indices were recorded with increasing the storage period. It is thought that with the increase in fruit storage time and the subsequent decrease in phenolic substances, the defense mechanism of the fruit is weakened. It has been shown that edible coatings create a modified atmosphere around the fruit and maintain carbon dioxide at levels higher than the normal state, which reduces both respiration and the oxidation reactions of phenols by reducing polyphenol oxidase activity. The edible coatings around the fruits also exert this effect by lowering the oxygen level around the crop (Lichtenthaler, 1987).

The results of the current study also showed that the peel and pulp flavonoid indices of fruits decline with increasing storage time. Flavonoids are found in a wide range of plant classifications, in particular in leaves, flowering tissues, and fruits (Gill et al., 2016). The absorption of free radicals and strong antioxidant activity are among the prominent properties of flavonoids (Larson, 1988).

Moreover, the results of the current study indicated that the firmness index of tested fruits increased with the rise of storage time. It is assumed that the prevention of weight loss and delayed fruit shrinkage through stomatal closure were some of the reasons for maintaining the fruit tissue firmness. During storage, fruits and vegetables undergo weight loss due to water discharge as vapor from the fruit's surface (Etemadipoor et al., 2019). Coatings create a barrier on the fruit surface and thereby help reduce water loss, moisture, and changes in the atmosphere around the fruit (Gill et al., 2016). Low oxygen levels and high carbon dioxide concentrations in the environment can potentially reduce the activities of pectin esterase and polygalacturonase and preserve fruit firmness during storage. A reduction in firmness loss can be attributed to reduced oxygen exchange because of a physical barrier by the coating that reduces respiration and, in turn, lowers the activity of hydrolyzing enzymes and delays softness (Etemadipoor et al. 2019). According to the data of the current study, more tissue firmness was observed in treatments with lower weight loss of tested fruits. This finding agrees with that reported by Etemadipoor et al. (2019) on the effect of an oleic acid edible coating containing cinnamon essential oil on the quality properties of guava fruits and with the study of Khaleghi et al. (2011) on the effect of hot water and sodium chloride treatments on the storage life and quality of three tomato cultivars. Mani et al. (2017) successfully utilized *A. vera* gel as the coating for African cedar fruits. The fruits coated with *A. vera* gel showed less weight loss, less firmness, and less acidity reduction than uncoated ones (Mani et al., 2017).

Based on the findings of this study, the soluble solid index rose with rising the storage time of guava fruits. As the amount of sugar increases, the amount of acidity decreases. Sridevi et al. (2018) reported that total sugar and TA contents were significantly lower (30%) in pomegranate seeds coated with *A. vera* gel during the first storage phase, The results obtained in the current study did not agree with the findings of Sridevi et al.'s (2018).

Additionally, the results of the current study revealed an increase in the pH index with an increase in storage time. Fruit extract pH indicates hydrogen ions and is important for enzymatic reactions and the activity of microorganisms, yeasts, and bacteria, but it does not affect the fruit taste (Larson, 1988). The results of the current study indicated an increase in pH over time that means increasing the time resulted in lower acidity and a higher pH of the extract, suggesting the inverse relationship between fruit pH and acidity.

In this study, the highest elevation (0.34 units or 50.75%) and the most reduction (0.00 or −0.17) of the TA index were recorded in the 2nd-week and 1th, 3th, and 4th-week treatments, respectively (Fig. 18). As an important parameter during storage, TA is directly associated with the concentrations of dominant organic acids. Acidity most probably decreases due to the biochemical changes in the fruit's organic compounds during the respiration process (Mani et al., 2017). Therefore, any treatment that causes delayed metabolism and aging of the crop can reduce the rate of TA changes during storage. Consistent with this result, there is evidence that the fruit's internal atmosphere changes by the semi-permeable coverage created by the edible coating, which reduces oxygen levels and increases carbon dioxide concentrations around the fruit (Cong et al, 2007). Therefore, the modified atmosphere delays the respiration rate, ripening, and aging process of the fruit (Corrales-Garcıa et al., 2004; Dragovicuzelac et al., 2007).

CONCLUSIONS

The present study investigated the effect of *A. vera* gel and hot water treatments on the reduction of chilling

injury in guava fruits. The use of *A. vera* gel and hot water treatments at various experimental levels and different periods led to increased values of such indices as the a* color index, fruit firmness, pulp and peel antioxidants, the ion leakage index, and the MDA index. The applied treatments reduced the indices of b and L colors, total chlorophyll, carotenoids, ascorbic acid, pulp and peel phenols, and pulp and peel flavonoids. It was concluded that the postharvest application of *A. vera* gel and hot water treatments could reduce chilling injury in guava fruits and result in better storage of fruits during storage.

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تأثیر تیمارهای آلوئه‌ورا و آب گرم بر حفظ کیفیت میوه گوآوا در شرایط انبار سرد

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چکیده – گواوا به‌دلیل ویژگی‌های فیزیکوشیمیایی دوره انبارمانی کوتاهی دارد. مطالعات متعددی در راستای حفظ کیفیت میوه گواوا و افزایش انبارمانی آن صورت گرفته است اما در حال حاضر یک روش بهینه برای آن وجود ندارد. این پژوهش به بررسی کاربرد پس از برداشت ژل آلوئه‌ورا و تیمار آب گرم بر کاهش سرمازدگی میوه گواوا پرداخته است. آزمایش به‌صورت فاکتوریل در قالب طرح کامل تصادفی روی میوه گواوا صورت گرفت. تیمارهای مورد استفاده شامل زمان انبارمانی در پنج سطح (صفر، ۷، ۱۴، ۲۱ و ۲۸ روز)، تیمار آب گرم در سه سطح (بدون آب گرم، ۴۰ و ۵۰ درجه سلسیوس) و ژل آلوئه‌ورا در سه سطح (صفر، پنج و ۱۰ درصد) و شاهد (آب مقطر، بدون ژل آلوئه‌ورا و بدون آب گرم) بودند. نمونه ها در دمای ۱±۷ درجه سلسیوس با رطوبت نسبی ۹۵٪ برای ۲۸ روز و سپس در ۲۴ درجه سلسیوس برای ۲۴ ساعت برای سازگاری با شرایط بازار ذخیره شدند. طبق نتایج به دست آمده، با گذشت زمان میزان شاخص رنگ*a افزایش و میزان شاخص‌های رنگ *L و *b کاهش یافت. از میان تیمارهای مورد آزمایش، تیمار ۵ درصد آلوئه‌ورا از طریق افزایش محتوای قند کل و کاهش درصد نشت یونی سبب حفظ کیفیت بهتر میوه گواوا شد. همچنین اثر متقابل تیمار آب گرم ۴۰ درجه سلسیوس و ۱۰ درصد آلوئه‌ورا با کاهش میزان تنفس و حفظ بیشتر سفتی در میوه اثرات مثبتی برجای گذاشت. مقادیر کلروفیل کل کلیه تیمارها در هفته چهارم کمترین مقدار را نسبت به تیمارهای مشابه در هفته های ۱-۳ داشت. افزایش دوره ذخیره سازی منجر به روند نزولی شاخص اسید اسکوربیک شد. حداقل مقدار کاروتنوئیدها و فلاونوئیدهای پوست در تیمار آب ۴۰ درجه سلسیوس و ۱۰ درصد آلوئه‌ورا مشاهده شد.. بیشترین مقدار آنتی‌اکسیدان پوست در تیمار بدون آب گرم و ۵ درصد آلوئه ورا، نشت یونی در تیمار بدون آب گرم و ۱۰ درصد آلوئه‌ورا و مالون دی‌آلدئید در آب ۴۰ درجه سلسیوس و ۵ درصد آلوئه‌ورا بدست آمد. نتایج این مطالعه نشان داد که استفاده از ژل آلوئه‌ورا و تیمار آب‌گرم باعث کاهش سرمازدگی میوه گواوا و ماندگاری بهتر میوه در طی زمان انبارمانی می‌گردد.