JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH 2021, VOL. 4 (SPECIAL ISSUE: FRESH-CUT PRODUCTS), 67-80



Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir



Citric acid and CaCl₂ extended the shelf life, maintained antioxidant capacity, and improved sensory attributes of fresh-cut kiwifruit

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ARTICLE INFO

Original Article

Article history:

Received 30 August 2021 Revised 8 October 2021 Accepted 9 October 2021 Available online 20 October 2021

Keywords:

Consumer acceptability

Food quality

Fresh-cut

Shelf life

DOI: 10.22077/jhpr.2021.4725.1243 P-ISSN: 2588-4883

E-ISSN: 2588-6169

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ABSTRACT

Purpose: Nowadays preserve fresh-cut fruits' quality is a challenge for their short shelf life and sooner undesired sensorial changes that cause unacceptability by consumers. This study aims to apply individual and combined citric acid and calcium chloride treatments in fresh-cut kiwifruit to improve its physicochemical properties, antioxidant content, and sensory acceptance. Research method: Kiwifruit cv. Hayward was disinfected and cut into slices of 10 mm thickness. Samples g were immersed for one minute in sterile distilled water (control), CaCl₂ 0.5%, Citric acid 1%, the citric acid and CaCl₂ combination 1.0:0.5 (citric acid: CaCl₂, %) and stored for 12 days at 5 °C. The physicochemical parameters, total antioxidant capacity, microbial quality, and consumer acceptability were measured during the storage. Findings: Individual treatments of CaCl₂ conserve color parameters increasing luminosity, and citric acid treatment kept the titratable acidity under storage conditions. However, the combination treatments delayed kiwifruit's maturation process, avoiding weight loss under storage conditions for 12 days at 5 °C. Besides, other parameters like color, pH, and titratable acidity presented significant differences compared with citric acid, CaCl₂ individual treatments and untreated fruits. Moreover, the citric acid and CaCl₂ combination maintained phenolic content and antioxidant capacity by inhibiting DPPH and ABTS radicals. Meanwhile, the untreated control kiwifruits presented the lowest antioxidant activity at the end of storage. Finally, the kiwifruit-combined treatment did not show microbial growth and gave higher consumers acceptability than the untreated fruit. Limitations: No limitations were encountered. Originality/Value: This study showed that citric acid, calcium chloride, and their combination are useful to extend freshcut kiwifruit shelf life while maintaining antioxidant capacity and sensorial acceptability.



INTRODUCTION

The main problem of the fresh-cut products industry is the high waste of biomass as a result of fruit susceptibility to tissue damages, browning, wilting and microbial spoilage (Rojas-Graü et al., 2009). The minimum processing of fruits has been defined as a combination of procedures, including washing, peeling, cutting or chopping, with the purpose to maintain the initial quality. It should be remembered that minimally processed fruits are living tissues, with an active metabolism; therefore, special attention to its processing is essential to assure a good quality (Caicedo Hoyos, 2021). However, good quality and safety can also be maintained and assured by applying food additives (Tapia et al., 2015). For this reason, it is necessary to define a process and treatments to minimized negative changes in the structure, sensory, nutritional, and microbiological properties of the minimally processed fruit.

Kiwifruit consumption offers health benefits and unique sensory and nutritional properties (Park et al., 2014). Its quality parameters present a minimum requirement of 12% soluble solids and a pulp firmness of 2 to 3 pounds of force (Cantin et al., 2011). In addition, kiwifruit is uncommon in many ways compared to other fruits, including its organoleptic properties: taste, color, aroma, and nutritional content. This fruit contained high levels of antioxidants such as vitamin C, carotenoids and polyphenols, and dietary fiber (Gammon et al., 2013; Sun-Waterhouse et al., 2009). On the other hand, minimally processed kiwifruit is highly perishable, and its minimal processing promotes physiological deterioration, biochemical changes, and degradation by spoilage microorganisms, which results in loss of color, flavor, and texture (Manzoor et al., 2021).

Different natural treatments can be applied to maintain the fresh-cut quality of kiwifruit and improve its shelf life. For this reason, calcium chloride (CaCl₂) solutions have been used to reduce loss of firmness and slow the ripening process in fruits. Besides, CaCl₂ treatments retard color changes and softening by retaining sugar and acid content (Antunes et al., 2005). On the other hand, many treatments can be used to reduce browning enzymatic reactions in fresh-cut products. However, organic acids can be helpful to delay polyphenol oxidase activity in sliced fruits like apples, peach, pear, mango, and others (Bhat et al., 2021). Therefore, this study aimed to demonstrate that the combination of citric acid and calcium chloride could be used to preserve the shelf life of fresh-cut kiwifruit under storage conditions, keeping nutritional content, delaying microbial growth without compromising consumer acceptability.

MATERIALS AND METHODS

Treatments application on fresh-cut kiwifruits

Kiwifruit cv. Hayward was purchased at a local fresh produce market in Hermosillo, Sonora, Mexico. They were selected at commercial maturity, uniform size and color, and free from visible surface defects. Fruits were washed and disinfected by immersion in a NaClO solution (200 mg/L) for 2 minutes, allowed to dry, and stored at 12 °C for 24 h. Disinfected kiwifruits were cut into slices of 10 mm thickness. Samples of 10 g were immersed for one minute in the following treatments solutions: sterile distilled water (control), CaCl₂ 0.5%, Citric acid 1%, the citric acid and CaCl₂ combination 1.0:0.5 (citric acid: CaCl₂, %). Once the treatments were applied to the kiwifruits, they were placed in transparent and sterile polyethylene-terephthalate (PET) trays and sealed under pressure with ethylene vinyl acetate films using a thermo sealer (Cryovac, Mexico City, Mexico). The samples were incubated at 4 ± 1 °C for 12 days.



Physicochemical attributes of fresh-cut kiwifruits

Treated kiwifruits were subjected to physicochemical tests. For this, 10 g of samples were homogenized in 50 mL of distilled water at pH 7 for 60 seconds and then filtered. Total soluble solids were measured using an Abbé digital refractometer (E-Inginst Electron Corp., Fujian, China), and the results were expressed as °Brix (Helrich, 1990). The pH was determined using an electronic pH meter directly on the homogenized samples (Fisher Scientific AB150, Ottawa, Canada). The titratable acidity was determined using a Mettler automatic titrator model DL21 (Columbus, OH, USA) and expressed as mg citric acid/100 g fresh weight (fw) (Lewis et al., 1995). The color parameters of kiwifruits pulps (L^* , a^* , b^*) were measured with a CR-400HS colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan). The physicochemical analysis was evaluated at 3, 6, 9, and 12 days after treatment. All measurements were made in triplicate.

Phenolic compounds and flavonoids content

Methanol extracts were obtained from kiwifruit pulp to quantify phenolic and flavonoid content following the methodology proposed by (Palafox-Carlos et al., 2012). First, fresh pulp samples (10 g) was homogenized (Homogenizer IKA, ultra Turrax, U.S.A) in 20 mL of methanol to 80% for 30 s at medium speed. Then, the samples were subjected to an ultrasonic bath (Bransonic Ultrasonic Co., Model 2210, Danbury, CT, USA) for 30 min at 2 °C and subsequently centrifuged at 1200 g at 4 °C for 15 min (Centrifuge Beckman Coulter, Allegra 64R, Calif. USA). This procedure was repeated twice with 10 mL of methanol (80%), and the volume of the supernatants was put together, filtered through Whitman No. 1 paper, and taken to a final volume of 50 mL with methanol (80%).

The total phenol content of each sample was determined by the Folin-Ciocalteau method (Singleton et al., 1999) with some modifications. For this, 75 μ L of Folin-Ciocalteu reagent [1:10], 15 μ L of the sample, and 60 μ L of Na₂CO3 at 7.5% were mixed and incubated for 30 min in darkness. The absorbance was measured at a wavelength of 765 nm in a microplate reader (Fluostar Omega, BMG Labtech Inc., Model Omega, Chicago, IL, USA). Total phenolic compounds were calculated using a calibration curve of gallic acid, and the results were reported as mg equivalent of gallic acid per 100g of fruit (mg GAE/100g fw). All samples were analyzed in triplicate.

The determination of total flavonoids was performed by the method described by Zhishen et al. (1999) with some modifications. For the assay, 100 μ L of the sample and 430 μ L of mixture A (1.8 mL of 5% NaNO2 with 24 mL of distilled water) were mixed and left to stand for five minutes. Subsequently, 30 μ L of AlCl₃ was added to 10% and left to stand for one minute. Then 440 μ L of mixture B (12 mL of NaOH 1M with 14.4 mL of distilled water) was added. From this reaction, 150 μ L were taken in triplicate, placed in the microplate reader (Fluostar Omega, BMG Labtech Inc., Model Omega, Chicago, IL, USA), and read at a wavelength of 496 nm. A calibration curve was developed with quercetin, and the results were expressed as mg of quercetin equivalents per 100g of fruit (mg QE/100g fw). All samples were analyzed in triplicate.

Antioxidant capacity by DPPH radical inhibition test

Total antioxidant activity was determined using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method. We first prepared the DPPH radical by diluting 1.97 mg of DPPH in 50 mL of pure methanol and adjusted it to an absorbance of 0.7. Then, 140 μ L of the adjusted radical and 10 μ L of the extract were added to a microplate and incubated for 30 min in darkness. The absorbance was read in a microplate reader at 518 nm (Fluostar Omega, BMG Labtech Inc.,

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Model Omega, Chicago, IL, USA). A standard curve was performed with Trolox, and the results were expressed in μ mol Trolox Equivalents (TE)/100 g of fresh weight (fw) (Robles-Sánchez et al., 2009). All samples were analyzed in triplicate.

Antioxidant capacity by the ABTS ⁺ assay

antioxidant capacity was The evaluated by the $ABTS^+$ [2,20-azino-bis (3ethylbenzothiazoline-6-sulfonic acid)] method. Firstly, we prepared ABTS⁺ radical in darkness by adding 19.3 mg of ABTS in 5 mL of distilled water. Then, 37.8 mg of K₂S₂O₈ was added in 1 mL of distilled water, and 88 µL of this solution was added to the 5 mL ABTS solution. This mixture was left to rest in the dark and at room temperature for 16 h. The radical was then adjusted into pure ethanol to an absorbance of 0.7. The adjusted radical (245 µL) and fruit extract (5 µL) was added to a microplate to start the reaction. It was left to rest in darkness for five min, and the absorbance was read at 754 nm (Fluostar Omega, BMG Labtech). Similarly, a calibration curve was elaborated with Trolox, and the results were expressed in µmol TE/100 g fw (Re et al., 1999). The determination was made in triplicate.

Microbiological analysis

Microbial growth in the fresh-cut kiwifruits treated with citric acid, calcium chloride, and their combination was measured at days 0, 3, 5, and 12 of storage at 5 °C. Ten grams of kiwifruit samples were diluted (1:9) in peptone water and homogenized for 10 minutes; subsequently, decimal dilutions were used for the microbial counting (Beirão-da-Costa et al., 2014). The mesophilic aerobic count was performed by incubating (48 h at 37 °C) the sample dilutions in plate count agar. Simultaneously, each sample dilution was incubated (24 h at 37 °C) in violet red bile agar to count coliform bacteria. Finally, the total mold and yeasts were counted in the sample dilutions using acidified potato dextrose agar incubated for 5 days at 25 °C. The colony-forming units of each type of microorganism were counted, and results were expressed in Log CFU/100g of fresh sample.

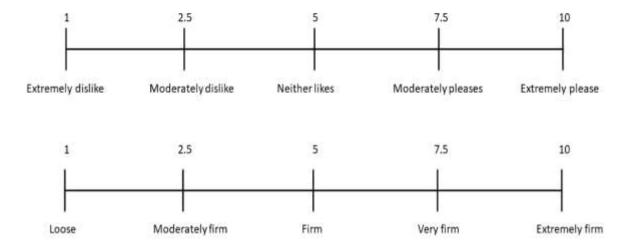


Fig. 1. Labeled hedonic scales that relate a numerical value to the level of liking and firmness of fresh-cut kiwifruit.



Sensory evaluation

The consumer acceptability of the treated fresh-cut kiwifruit was carried out using a consumer-level test (Fig. 1) with 30 panelists who were not aware of the treatments (Schutz & Cardello, 2001). The attributes evaluated were the global and the firmness liking level. The samples were assessed at 0, 3, 6, and 9 days of storage. Two 5-point hedonic scales were used, where for the overall liking test, 1 was extremely disliked, and 10 extremely pleased, and for the firmness level test, 1 was not firm, and 10 extremely firm.

Statistical analysis

All experiments were conducted in a completely random design. Data were subjected to an analysis of variance (ANOVA), and the means were compared using the Turkey-Kramer test to estimate significant differences among the treatments (p<0.05). This analysis was performed using the Statistical Number Crunch System Software (NCSS Statistical Software, Kaysville, UT, USA).

RESULTS

Total soluble solids

The total soluble solids (expressed as °Brix) of fresh-cut kiwifruits stored at 5 °C for 12 days are shown in Figure 2A. This parameter includes the sugar content in fruits related to their maturation rates. The results show that °Brix increased during storage for all the treatments as was expected. In addition, this could be due to the soluble solid concentration caused by the fruit water loss. At the end of the storage, a higher increment in °Brix was observed in untreated kiwifruits. The °Brix of CaCl₂, citric acid, and the combination treatments slightly changed during storage, suggesting that the treatments could delay the maturation process. A significant effect on preserving total soluble solids was observed with the CaCl₂ treatment compared to the control group (p<0.05).

Weight loss

The control and treated samples' weight loss was measured over the storage time (Fig. 2B). All fruit samples lost weight at the end of the storage period. After 6 days, the control fruits began to lose weight slightly; at day, 9 control fruits lost less than 3% and presented a smaller amount of weight loss than the rest of the treatments in this time. However, on day 12, weight loss in the control fruits was higher (more than 6%) compared to the combination and CaCl₂ treatments (2.8%). The combination of citric acid and CaCl₂ caused a weight loss from day 3 of storage, but it was maintained with minor changes for the rest of the period. The treatment with CaCl₂ increased the weight loss at the end of the period, but it best preserved the fruits' weight. Treatment with citric acid at the end of the period presented a weight loss similar to the control samples.

pН

The pH of all samples increased gradually over the storage period (Fig. 2C), even when no significant differences were observed among treatments. The control fruit showed higher values than the other samples since the sixth day. The treatments delayed the increase in the pH values from day 6 and maintained the pH most stable during storage compared to control samples. The lower pH at day 12 was observed in fruits treated with the combination of citric acid and CaCl₂.

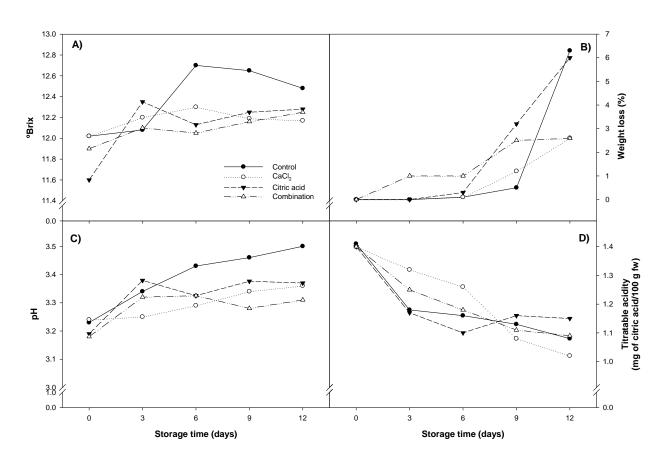


Fig. 2. Physicochemical analysis A) Total soluble solids (°Brix), B) Percentage of weight loss, C) pH and D) Titratable acidity content of untreated (control) and treated kiwifruits (CaCl₂, Citric acid, combination of CaCl₂ + Citric acid) stored for 12 days.

Titratable acidity

Titratable acidity of samples was measured and expressed as citric acid content (Fig. 2D). All the treatments presented a similar titratable acidity on day zero. The acidity decreased in all treatments during the 12 days of storage (p<0.05). The citric acid treatment kept the titratable acidity more stable after day 3 of storage, and on day 12, it presented the highest values compared to the other treatments, but no statistical difference was observed. The acidity decreased slightly in the fruits treated with CaCl₂ and the combination from days 0 to 3 and from 3 to 6 days compared to the other treatments. However, from day 9 to 12, the fruits treated with CaCl₂ presented lower titratable acidity values while the combination showed similar values to the control at the end of storage, but no significant difference was observed.

Color

The color of fresh-cut kiwifruits was affected by all treatments and storage time. The color parameters L, a^* , and b^* were measured in the kiwifruit pulps (Fig. 3). The storage period influenced the luminosity of the samples, observing that over time the L values decreased with all the treatments, which means that the samples darkened (Fig. 3A). At the end of the storage, the control and the fruits treated with citric acid and the combination showed similar values of L, while the treatment with CaCl₂ maintained a higher luminosity, but no significant differences were observed.

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Values of a^* indicate the color of the sample between green (more negative values of a^*) and red (more positive values of a^*). At the beginning of storage, all the fruits presented similar values of a^* while over time, these values increased, losing little of the initial intense green color (Fig. 3B). The control fruits preserved their green color from the beginning until day 9 of storage, but from that day until day 12, they presented a drastic increase in the value of a^* . The fruits treated with CaCl₂ and the combination showed a slight increase in the value of a^* through storage, indicating a more reddish coloration. At the end of the period, fruits treated with CaCl₂, citric acid, and their combination showed similar values of a^* .

Similarly, fruits values of b^* (+b indicate yellow, -b indicate blue) in all treatments were affected by the storage time. The control samples showed a lower decrease in b^* values through the storage period, and on day 12 had higher b^* values indicating yellow colorations (Fig. 3C). Although no significant differences were found among treatments in any day of storage, the treated fruit showed higher variations in b^* values. At the end of the storage, the CaCl₂ samples exhibited similar b^* values to control, and the lower values were observed in the combination treatment.

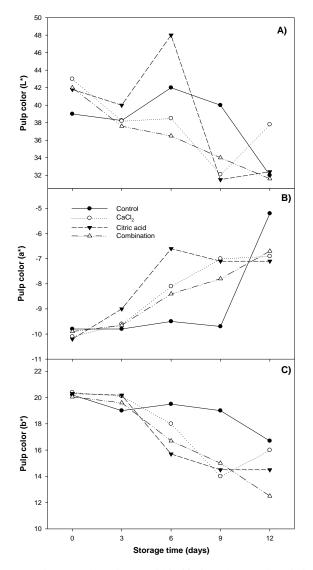


Fig. 3. Color analysis of untreated (control) and treated kiwifruit pulp (CaCl₂, Citric acid, combination of CaCl₂ + Citric acid) stored for 12 days. A) Pulp color (L^*), B) Pulp color (a^*), C) Pulp color (b^*)



Phenolic content and antioxidant activity of treated kiwifruits

The phenolic and flavonoids content was measured in untreated and treated fresh-cut kiwifruits (Figure 4). The phenolic content in all samples was decreased during storage time (Fig. 4A) (p<0.05). The control samples showed the lowest phenolic content at day 0, and only a slight decrease was observed over time. The combination of citric acid and CaCl₂ caused a noticeable reduction in phenolic content on day 3, but these values were maintained until day 12, being the treatment with the highest phenolic content value at the end of storage. The samples treated with citric acid showed a slight decrease in phenolic content in the first 6 days; however, from day 6, a more significant decrease was observed until the end of the storage. In addition, the CaCl₂ samples showed a decrease in phenolic content, obtaining similar values to citric acid samples at day 12. No statistical difference was found among treatments at the end of the storage. Likewise, the flavonoid content was decreased through the storage period in all samples (Fig. 4B). The control fruits showed a higher decrease in most of the evaluation days (p<0.05). The combination treatment was the most effective to preserve the flavonoid content in fresh-cut kiwifruits for 12 days (p<0.05). The CaCl₂ and citric acid samples showed similar behavior between them (p>0.05) and were different to control and combination (p<0.05).

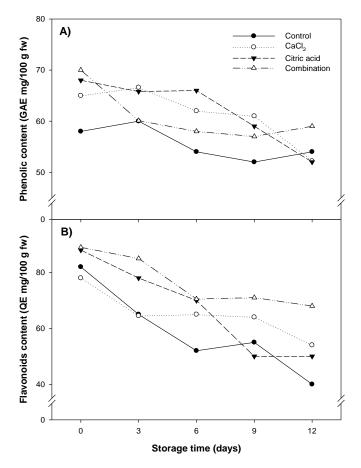


Fig. 4. A) Phenolic and B) Flavonoid content of untreated (control) and treated fresh-cut kiwifruit (CaCl₂, Citric acid, combination of CaCl₂ + Citric acid) stored for 12 days.



Regarding the antioxidant capacity, the DPPH and ABTS assays revealed similar results (Fig. 5). All samples showed a decrease in antioxidant capacity caused by the storage time (p<0.05). In the DPPH assay, the control samples presented a higher reduction from day 3 until the end of the storage time, showing the lowest antioxidant values at day 12 compared to the other treatments. The citric acid and the combination samples showed a slight decrease in the antioxidant capacity through time, but at the end of the storage, both treatments were more effective in preserving fresh-cut kiwifruit's antioxidant capacity (p<0.05). On day 12, the CaCl₂ samples showed higher antioxidant capacity than control but lower than citric acid and the combination fruits (p<0.05). In the ABTS assay, the control samples maintained the antioxidant capacity for the first 6 days of storage; from this day, a decrease in antioxidant capacity was observed until the end of the storage. The control samples had the lowest antioxidant capacity (p < 0.05). The CaCl₂ fruits maintained the antioxidant capacity measured by ABTS assay for the first 6 days; then, a slight decrease was observed from day 9 to day 12. These samples presented the highest antioxidant capacity at the end of the storage (p < 0.05). The citric acid showed the highest antioxidant capacity the first 3 days of storage and on day 12 presented similar values to the combination.

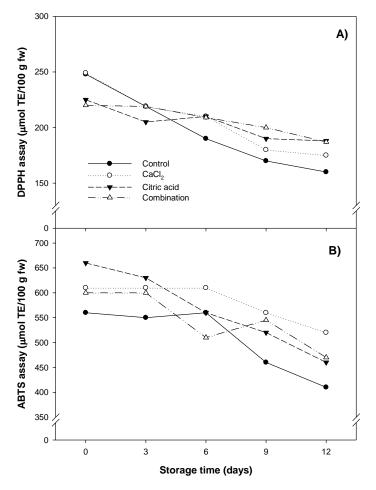


Fig. 5. Antioxidant capacity of untreated (control) and treated fresh-cut kiwifruit (CaCl₂, Citric acid, combination of $CaCl_2 + Citric$ acid) stored for 12 days. A) DPPH radical inhibition and B) ABTS radical inhibition.



Microbial counts in treated kiwifruits

The aerobic mesophiles, total coliforms and fungi, and yeast of untreated and treated fresh-cut kiwifruits were counted every three days for 12 days of storage at 5 °C. The microbiological analysis showed the absence of total coliforms in all the samples during the storage period. Likewise, no aerobic mesophilic counts were detected on the control samples and those treated with CaCl₂ for 9 days. On day 12, the control fruits showed a count of 1690 CFU/g of aerobic mesophiles, and the fruits treated with CaCl₂ showed 1340 CFU/g. The samples treated with the combination and citric acid did not reveal the presence of mesophiles during the 12 days of storage. No fungi and yeasts were detected in the control samples for 6 days, while <365 CFU/g was counted on days 9 and 12. Fungi and yeasts counts could not be detected in the samples treated with CaCl₂, citric acid, and the combination for 9 days of storage, while on day 12, the counts were lower than 550 CFU/g.

Sensory evaluation of treated kiwifruits

Table 1 shows the consumer global and firmness liking level of untreated and treated freshcut kiwifruits stored a 5 °C for 9 days. The storage time influenced the decrease in the consumer liking level for all samples (p<0.05). The consumers perceived as acceptable the general sensory attributes of the kiwifruits treated with the selected concentration of these additives and the control fruits within a moderate liking level. No significant difference in global liking level was observed among treatments at day 0 and at the end of the storage. The fruits treated with CaCl₂ and citric acid combined showed a slightly greater liking level at day 9 than the other treatments (p<0.05).

The texture is the limiting factor of kiwifruit quality, and firmness is its characteristic parameter. On day 0, the fruits treated with $CaCl_2$ obtained the highest firmness level, followed by the combination and citric acid (p<0.05). The storage time, as expected, decreased the firmness perception level of consumers. Consumers identified at the end of the storage the $CaCl_2$ fruit as firm, while the fruits treated with the combination were perceived as less firm. The control samples and those treated with citric acid were moderately firm, obtaining the lowest firmness values (p<0.05). These results show that citric acid and $CaCl_2$ alone or combined could be used as fresh-cut fruit additives.

	Treatments			
Storage time (days)	Control	CaCl ₂	Citric acid	$CaCl_2 + Citric acid$
	Global liking level			
0	7.5ª	6.8ª	6.4 ^a	7.3 ^a
3	6.3 ^b	6.9 ^{bc}	5.5 ^a	6.7 ^b
6	5.3ª	6.1ª	4.79 ^a	6.2ª
9	4.3 ^a	5.1ª	3.99 ^a	5.2ª
	Firmness liking level			
0	4.2ª	5.9 ^b	4.6 ^a	5.6 ^{ab}
3	3.6ª	5.2 ^b	3.7ª	5.6 ^b
6	3.2ª	5.5 ^b	2.4 ^a	4.3 ^{ab}
9	2.7ª	4.8 ^b	2.6 ^a	4.3 ^b

Table 1. Global and firmness liking level of untreated (control) and treated fresh-cut kiwifruits (CaCl ₂ , Citric
acid, combination of CaCl ₂ + Citric acid) stored at 5 °C for 9 days

*Different letters mean significant differences among treatments in the same row.



DISCUSSION

Physicochemical kiwifruits properties like color and water content can be considered primary factors that affect consumer acceptability. Therefore, keeping these quality values improves the acceptance level of fresh-cut kiwifruit (Manzoor et al., 2021). At the end of storage time, individual treatments with CaCl₂ conserved color and weight loss parameters compared with combination and untreated control fruits. Furthermore, only citric acid treatments decreased titratable acidity parameters in kiwifruit slices. In this context, related findings were observed in kiwifruits conserved in fresh-cut treatments with ascorbic and citric acid avoiding undesired browning reactions and weight loss under storage conditions (Bhat et al., 2021). On the contrary, citric acid and combination treatments affect luminosity, decreasing b^* values presenting yellow pigments in kiwifruit slices. On the other hand, all the treatments increased pH and total solid values; these findings showed that sugar content in fruits was preserved, delaying maturation processes during its shelf life.

Phenolic and flavonoid compound content of all kiwifruits treatments decreased significantly under storage days. The decomposition values of bioactive compounds in kiwifruit were strongly sensitive when minimal processes were applied to whole fruits (Kim et al., 2018). Fresh kiwifruits contain considerable levels of chlorophyll, lutein, β -carotene, procyanidin, catechin, and chlorogenic acid (Siddique et al., 2021). Meanwhile, temperature conditions were at 5 °C; kiwifruits were exposed to a different environment with light and pH modifications related to affect the structure of phytochemical compounds in fresh-cut fruits during 12 days of storage. For this reason, in all treatments, antioxidant activity was preserved during the first 6 days of storage.

In sensorial acceptance terms, CaCl₂ and combination treatments presented higher acceptable values in firmness parameters than untreated fruits. CalCl₂ can contribute to stabilizing the kiwifruits cell wall by forming calcium pectates, thus helping to reduce the solubilization of the pulp and therefore keep the fruit firm (Antunes et al., 2005). Besides, in combination with citric acid as an additive, it helps preserve the overall acceptability and keeps the pH levels low; therefore, most spoilage microorganisms cannot grow under these conditions in treated kiwifruits during storage conditions.

CONCLUSION

The use of additives such as organic acids and texturizing compounds significantly lengthens the shelf life of minimally processed kiwifruit, improves sensory attributes, and retards microbial deterioration. The use of calcium chloride solution and its combination with citric acid maintains the quality of minimally processed kiwis for up to 12 days under storage conditions at 5 °C, without affecting their sensory attributes. However, more combinations with other additives that help preserve antioxidant activity should be explored to enhance the shelf life of fresh-cut products in nutritional and food safety terms.

Conflict of interest

The authors have no conflict of interest to report.



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