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Physicochemical changes, phenolic compounds and antioxidant capacity in nanche (*Byrsonima crassifolia* L. Kunth) fruits during refrigerated storage

Cambios fisicoquímicos, compuestos fenólicos y capacidad antioxidante en frutos de nanche (*Byrsonima crassifolia* L. Kunth) durante el almacenamiento en refrigeración

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ABSTRACT

Nanche (*Byrsonima crassifolia* L. Kunth) is a fruit with significant potential due to its commercial value and health benefits. However, postharvest fruits exhibit a short shelf life, limiting their marketability and consumption. In this context, the present study aimed to evaluate physicochemical changes, total phenolic content, and antioxidant capacity using refrigeration systems. Two treatments were established: fruits stored at 22 °C (Treatment 1, T1) and fruits stored in a climate chamber at 8 °C (Treatment 2, T2) for 12 days. Evaluations were conducted on days 0, 3, 6, 9, and 12. Refrigeration was effective in reducing physiological and physicochemical parameters (weight loss, firmness, color, °Brix, and citric acid content). Regarding total phenolic compounds, T1 and T2 showed values of 97.09 and 115.88 mg GAE/100 g f. w., respectively. Moreover, antioxidant capacity was observed in nanche fruit. Therefore, implementing a postharvest management system using refrigeration temperatures may extend the shelf life of this fruit while preserving its commercial value and health-promoting properties.

KEY WORDS: *Byrsonima crassifolia* (L.) Kunth, Malpighiaceae, storage, refrigeration, fruit quality.

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R E S U M E N

El nanche (*Byrsonima crassifolia* L. Kunth) es una fruta con potencial debido a su valor comercial y efecto benéfico para la salud. Sin embargo, los frutos después de cosechados presentan una vida de anaquel corta, limitando su comercialización y consumo. En ese contexto, el presente trabajo tiene como objetivo evaluar los cambios fisicoquímicos, la concentración de fenoles totales y la capacidad antioxidante mediante el uso de sistemas refrigeración. Por lo que, se formaron dos tratamientos; frutos almacenados a 22 °C Tratamiento 1 (T1) y frutos almacenados en una cámara climática a 8 °C Tratamiento 2 (T2) por 12 días. Las evaluaciones se realizaron los días 0, 3, 6, 9, 12. El uso de la refrigeración fue efectiva, reduciendo parámetros fisiológicos y fisicoquímicos (pérdida de peso, firmeza, color, °Brix y % de ácido cítrico). Para el caso de los compuestos fenólicos totales T1 y T2 presentaron valores de 97.09 y 115.88 mg EAG/100 g. p. f., respectivamente. Además, se observó capacidad antioxidante en el fruto de nanche. En ese sentido, establecer un sistema de manejo poscosecha mediante la utilización de temperaturas de refrigeración puede incrementar la vida útil de este frutal, conservando su valor comercial y sus propiedades benéficas a la salud.

P A L A B R A S C L A V E : *Byrsonima crassifolia* L. Kunth, Malpighiaceae, almacenamiento, refrigeración, calidad del fruto.

Introduction

Nanche (*Byrsonima crassifolia* L. Kunth) is a fruit belonging to the Malpighiaceae family. It is a small drupe with a strong aroma, a bittersweet and slightly oily flavor. During ripening, the fruit transitions in color from green to yellow, displaying orange hues until senescence. It can be consumed fresh or processed into juices, liqueurs, ice creams, preserves, dehydrated products, among other forms (de Araujo *et al*., 2018; San-Martín-Hernández *et al*., 2023). Several nutraceutical properties have been attributed to this fruit, including the presence of antioxidants, vitamin C, and dietary fiber. Thus, it represents an excellent source of vitamins and minerals, offering health benefits to consumers, such as the prevention of chronic degenerative and cardiovascular diseases (Sousa & de Souza 2020; Santana *et al*., 2023). In 2023, 2,096 hectares of nanche trees were cultivated in Mexico, achieving an average yield of 10,284 t ha^{-1}. Of the total production, the state of Nayarit contributed approximately 1,153 tons, equivalent to 11.21 % of the national production (SIAP, 2024). However, in Nayarit, postharvest management practices for nanche are not yet well defined. After harvest, fruits undergo rapid deterioration (< 5 days at 20 °C), especially in humid tropical regions, where environmental conditions accelerate decomposition,

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limiting their commercialization and consumption (Medina-Torres *et al*., 2012; Rivas-Castro *et al*., 2019). Despite being one of the main nanche-producing states in Mexico (SIAP, 2024), studies conducted in Nayarit have primarily focused on the selection and outstanding morphological characterization of this fruit (Agredano-De la Garza *et al*., 2021b; López-Guzmán *et al*., 2023). However, very few studies have addressed its postharvest quality and the presence of functional metabolites (Medina-Torres *et al*., 2015; 2021), which suggests that nanche may be considered a neglected crop with an undefined commercial focus (Baldermann *et al*., 2016; Porto *et al*., 2020). Some reports merely suggest that the fruits should be packed in small containers and stored at temperatures between 9 and 13 °C, under which they can maintain acceptable quality conditions for 10 to 12 days (Morton, 2004; Duarte, 2011). In this regard, studies conducted in Brazil by Mata *et al*. (2016) and Neves *et al*. (2015) evaluated nanche fruits stored at 12 °C and reported an increased shelf life of 10 to 12 days, consistent with previous findings. Therefore, the use of refrigeration during the postharvest storage of nanche is necessary to develop an optimal strategy for fresh preservation and to improve accessibility in regions where fresh fruit is not readily available (Rivera-Correa *et al*., 2022a). Given this background, establishing a postharvest management system using refrigeration temperatures would help extend the shelf life of this fruit, which holds potential due to its commercial value and nutritional properties. In this context, the present study aims to evaluate physicochemical changes, total phenolic content, and antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays during refrigerated postharvest storage.

Materials and Methods

Experimental site and plant material

For this research, nanche fruits (*Byrsonima crassifolia* L. Kunth) at physiological maturity were used, following the criteria of Martínez-González *et al.* (2017). The fruits were harvested from a commercial orchard located in the ejido “El Rincón,” in the municipality of Tepic, Nayarit (21° 34′ 483” N, 104° 56′ 215” W). Fruits were placed in plastic crates and transported to the Special Analysis Laboratory at the Unidad de Tecnología de Alimentos of the Universidad Autónoma de Nayarit. The fruits were selected to eliminate those with mechanical, physical, or phytopathological damage. Subsequently, they were washed with a 1 % sodium hypochlorite solution to prevent microbial proliferation.

Experimental design

The fruits were divided into two batches (90 fruits per batch) to establish two treatments: storage at 22 °C (T1) and storage at 8 °C (T2), both under 90 % relative humidity (RH). Fruits were stored in a climatic chamber (Climacell®). Evaluations were conducted on days 0, 3, 6, 9, and 12. A completely randomized design was used, with one fruit as the experimental unit and six replicates per treatment. Data were subjected to analysis of variance, and means were compared using Tukey's test at a significance level of *P ≤ 0.05*, employing the Statistical Analysis System software (SAS® v. 9.2) (Castillo, 2011).

Evaluated variables

Sample preparation. For the determination of total soluble solids, total phenolic compounds, and antioxidant activity (DPPH, ABTS, and FRAP), 1 g of fresh nanche pulp was homogenized in an Ultraturrax T25 IKA® with 10 mL of distilled water and centrifuged at 18,510 g at 4 °C for 30 minutes (Hermle Z326K). The aqueous phase was recovered for analysis.

Cumulative weight loss. Measured using a digital scale (Scout Pro, OHAUS®), and reported as a percentage (%) of the initial fruit weight relative to values recorded at each evaluation time.

Firmness. Assessed with a penetrometer (Force Gauge model GY-4) using an 8 mm diameter probe. Results were expressed in Newtons (N).

Total soluble solids. Measured by placing a few drops of juice on a digital refractometer (HANNA HI 96801), previously calibrated with distilled water. Results were expressed in degrees Brix.

Titratable acidity. Determined according to AOAC official methods (2005) by volumetric titration with sodium hydroxide (NaOH) using phenolphthalein as an indicator. Results were expressed as a percentage of citric acid.

Color. The peel color components were measured: brightness or reflected light (L) (0= pure black, 100= pure white), hue angle (h) (0= reddish-purple, 90= yellow), and chromaticity (C, intensity from gray to pure chromatic color), using a colorimeter CR-400 (Konica Minolta Sensing, Inc., Tokyo, Japan) following the method of Neguerula (2012).

Total phenolic compounds. Determined according to Stintzing *et al*. (2005). Briefly, 0.5 mL of the sample was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water), allowed to react for 5 minutes, then 2 mL of 7.5 % sodium carbonate (w/v) was added. The mixture was incubated at room temperature for 30 minutes, and absorbance was read at 765 nm using a microplate reader (Power Wave XS, Biotek). Results were expressed as mg gallic acid equivalents (mg GAE/100 g.f.w.).

DPPH assay. Determined using the method described by Brand-Williams *et al*. (1995). A DPPH• solution (7.4 mg/100 mL in 80 % ethanol) was stirred for 60 minutes, then diluted with 80 % methanol until an absorbance of 0.70 (±0.02) at 520 nm was obtained. For the assay, 50 μL of sample was mixed with 250 μL of DPPH solution in a 1.5 mL Eppendorf tube and incubated in the dark for 30 minutes. Absorbance was measured at 520 nm (Power Wave XS, Biotek). Antioxidant activity was expressed as mg ascorbic acid equivalents (mg AAE/100 g. f. w.).

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ABTS assay. Quantified following the method of Re *et al*. (1999). Two solutions were prepared: 7 mM ABTS and 2.45 mM potassium persulfate in distilled water. Equal volumes 1:1 (v/v) of both solutions were mixed and incubated in the dark at 23 ± 1 °C for 16 hours under constant agitation. The resulting ABTS•+ radical was diluted with ethanol (20 %) until an absorbance of 0.70 (±0.02) at 754 nm was obtained. For the assay, 10 µL of sample was added to 490 µL of ABTS solution, incubated for 7 minutes, and absorbance was read at 734 nm. Results were expressed as mg ascorbic acid equivalents (mg AAE/100 g fresh weight).

FRAP assay. Determined following the method of Benzie & Strain (1996), which evaluates the ability of compounds to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. In a 1.5 mL Eppendorf tube, 25 µL of sample was mixed with 63 µL of 0.2 M phosphate buffer (pH 6.6) and 63 µL of 1 % potassium ferricyanide ($K_3Fe(CN)_6$). After vortexing, the mixture was incubated at 50 °C for 30 minutes. Then, 63 µL of 10 % trichloroacetic acid was added, vortexed for 1 minute, and a 126 µL aliquot of the supernatant was transferred to another Eppendorf tube. Subsequently, 126 µL of distilled water and 25 µL of 0.1% ferric chloride ($FeCl_3$) were added. The mixture was vortexed, and the absorbance was measured at 700 nm. Results were expressed as mg ascorbic acid equivalents (mg AAE/100 g fresh weight).

Results and Discussion

Weight loss decreased in both treatments (T1 and T2) throughout the storage period. However, cumulative weight loss was significantly lower in fruits stored at 8 °C (T2) compared to those stored at 22 °C (T1), as shown in Figure 1A. The lower weight loss observed in fruits stored at 8 °C may be attributed to reduced water loss associated with a lower respiration rate at this temperature (Weber, 2020). Similar findings have been reported for other fruits such as mamey (Alia-Tejacal *et al*., 2020), apple (Ulloa *et al*., 2015), and pineapple (Valero & Serrano, 2010), where lower storage temperatures were correlated with reduced weight loss.

Fruit firmness in treatments T1 and T2 decreased gradually over the storage period. However, T1 fruits showed significantly lower firmness compared to T2, reaching 4.3 N by day 9 of storage (Figure 1B). This behavior is considered normal, as softening is a natural process in plant tissues during ripening or senescence due to the activity of pectolytic enzymes responsible for the degradation of the cell wall (Wang *et al*., 2018). In this regard, the use of refrigeration and varying storage durations can influence the reduction in fruit firmness, likely by suppressing the activity of cell wall-degrading enzymes (Galvis *et al*., 2002). These results are consistent with those reported by dos Santos *et al*. (2018), who observed firmness values between 3.09 and 4.76 N and reported that nanche fruit tends to become less firm as it ripens.

Both treatments, T1 and T2, showed a similar trend in total soluble solids, with values increasing over the storage period. Statistically, T1 fruits exhibited higher total soluble solids, with an average of 15.66 °Brix during postharvest storage, while T2 fruits recorded lower values, averaging 11.73 °Brix (Figure 1C). According to Jeronimo & Kanesiro (2000), the increase in total soluble solids may be due to the transformation of stored reserves during fruit development into

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sugars. Rivera-Correa *et al*. (2022b) also reported that soluble solids increase during nanche fruit ripening and suggested that refrigeration temperatures may influence physicochemical changes such as soluble solids content. Similarly, Carvalho & do Nascimento (2016) reported values ranging from 12.20 to 17.72 °Brix in nanche fruits, comparable to the values obtained in this study.

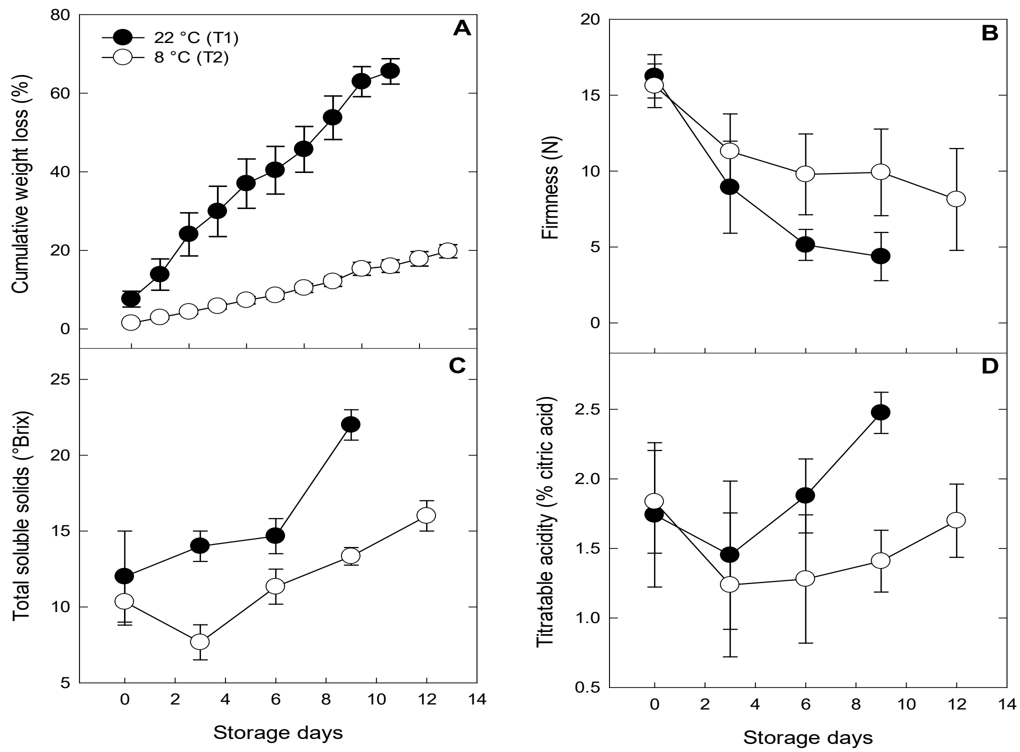

Figure 1. Cumulative weight loss (A), firmness (B), total soluble solids (C), and titratable acidity (D), each point represents the mean of six observations and its standard error.

Titratable acidity (% citric acid) decreased in T1 and T2 treatments on day 3 of storage but increased again in the following days, showing a rising trend of titratable acidity over time. T1 fruits had significantly higher acidity (1.88 % citric acid) compared to T2 fruits, which averaged 1.49 % (Figure 1D). Differences in acidity % between treatments may be attributed to lower metabolic activity at reduced storage temperatures, which can limit the synthesis of certain compounds, including organic acids (Valero & Serrano, 2010). Costa *et al*. (2012) reported acidity values ranging from 0.95 % to 1.08 % in nanche fruits from the coastal region of Maranhão. In contrast, Carvalho *et al*. (2008) characterized nanche fruits from a germplasm bank at Embrapa Amazônia

Oriental and found a higher average acidity of 2.36 % citric acid equivalent. Variations in acidity concentrations may be influenced by factors such as plant genetics, regional climate, and fruit ripeness at harvest, which could explain the differences observed in this research (Chitarra & Alves, 2001).

Color (L, C, h), luminosity (L) decreased in both treatments over the storage period, however, fruits of T1 showed significantly lower luminosity values, meaning they appeared less bright than fruits of T2. The behavior of Chroma (C) remained relatively constant in both treatments, although T1 fruits exhibited a significant decline by day 9 of storage. Regarding hue angle (h) (Figure 2), fruits in T2 maintained a yellow coloration throughout the evaluation period and recorded significantly higher hue angle values, in contrast, T1 fruits progressively shifted toward a brown color by the end of the storage period. Therefore, on average, the color of the fruits of T1 and T2 was bright opaque yellow (h = 79.5, C = 45.92, L = 66.87 and h = 83.67, C = 49.53, L = 70.55), respectively. Nanche fruits typically display a yellow coloration due to the presence of carotenoids. Although these compounds are relatively stable during storage, they are susceptible to degradation through dehydration and oxidative reactions (Rodríguez-Amaya, 1999; Rivas-Castro *et al*., 2019), which can impair the fruit's ability to reflect or absorb light. Furthermore, Rivera-Correa *et al*. (2022b) reported that low-temperature storage can slow changes in color parameters (LCh), a finding that aligns with the present study. Fruits stored under refrigeration (T2) exhibited slower changes in color attributes, this coincides with the findings of this study, as the fruits of T2 showed changes in these parameters.

The concentration of total phenolic compounds in T1 fruits showed an initial decrease from day zero to day two, followed by an increase to 97.09 mg GAE/100 g.f.w. by day six. This was subsequently followed by another decrease to 80.86 mg GAE/100 g.f.w. by day nine of storage. In contrast, T2 fruits exhibited an increase in phenolic content on days three and six, followed by a decline to 79.54 mg GAE/100 g. f. w. on day nine, and a subsequent increase to 115.88 mg GAE/100 g, f. w. by day 12. In summary, T2 fruits showed an opposite trend to T1 fruits, with the highest phenolic concentration occurring on day twelve. However, phenolic content in both treatments did not present significant differences by day nine (Figure 3H). In this sense, the phenolic levels reported in this study were higher than those documented by Carvalho & do Nascimento (2016) in four out of five nanche genotypes ripened at ambient temperature. However, they were lower than those reported by Belisário *et al*. (2020), who found phenolic concentrations of 165.2 mg GAE g^{-1} on day zero and 133.0 mg GAE g^{-1} on day sixteen during storage at 12 ± 1 °C, as well as those reported by Torres *et al*. (2024), ranging from 2.73 to 3.64 mg GAE g^{-1}. The decline in total phenolic concentrations during postharvest storage may primarily be attributed to the natural processes of fruit ripening and senescence. Several studies have shown that most phenolic compounds exist in the form of phenolic acids, which are prone to oxidation and enzymatic degradation as cellular metabolism progresses (Belisário *et al*., 2020). Nevertheless, phenolic compounds also include carotenoids and flavonoids, which can accumulate in nanche fruits as maturity indicators and as attractants for seed dispersers. These may contribute to the phenolic increase observed in T1 fruits on day 12 (Irías-Mata *et al*., 2018; Agredano-de la Garza *et al*., 2021). This transformation not only alters the fruit's phytochemical profile but may also compromise its antioxidant potential and thus its nutraceutical value (López *et al*., 2022). In this context, implementing cold storage

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strategies has become a key practice to mitigate such effects. By maintaining fruits at controlled temperatures around 12 ± 2 °C, metabolic activity is significantly slowed, reducing the degradation of bioactive compounds and minimizing nutritional losses (Chitarra & Chitarra, 2005; Belisário & Coneglian, 2013). Therefore, the refrigeration conditions used in this study contribute not only to preserving the fruit's functional quality but also to extending its shelf life and commercial viability.

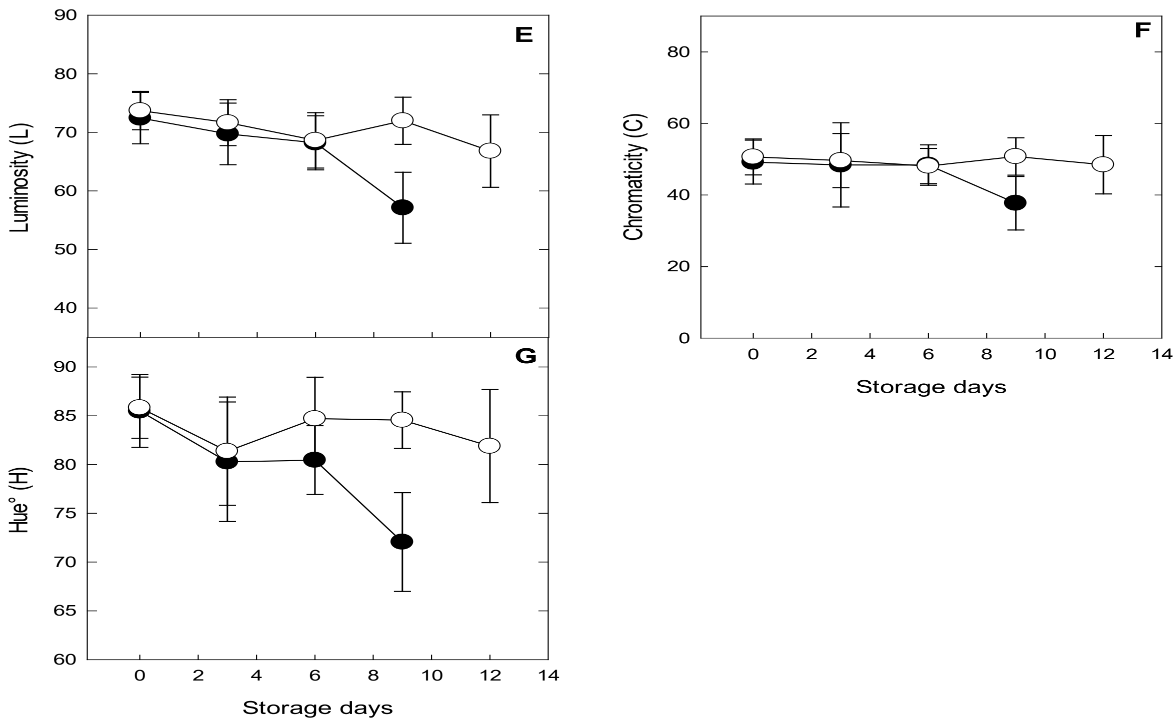

Figure 2. Luminosity (L), chromaticity (C), hue angle (h), each point represents the mean of six observations ± standard error.

The antioxidant capacity of nanche fruit is attributed to the presence of vitamins, phenolic compounds, and carotenoids, among others (Almeida *et al*., 2024). Thus, it is essential to assess antioxidant capacity using multiple methods, with DPPH, ABTS, and FRAP being among the most common, there is no universal method, as the reagents respond differently depending on the type of antioxidant. For instance, ABTS detects both hydrophilic and lipophilic compounds, while DPPH is limited to organic media and less polar compounds (Ramful *et al*., 2011; Almeida *et al*., 2011). In this study, the highest values were obtained with the DPPH method (Figure 3I), where both T1 and T2 treatments exhibited a decreasing trend in antioxidant capacity from 282.65 to 249.06 and

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from 267.27 to 243.01 mg AAE/100 g.f.w., respectively, with statistically significant differences. Regarding the ABTS method (Figure 3J), T1 fruits maintained relatively stable values throughout storage (from 80.81 to 78.42 mg AAE/100 g.f.w., whereas T2 fruits exhibited lower and decreasing values from 66.14 to 54.32 mg AAE/100 g.f.w., being statistically different from T1. This behavior may be linked to ripening processes in the fruit, where compounds such as phenolics, carotenoids, and flavonoids undergo oxidation during storage (Belisário *et al*., 2020). It is important to consider that fruits contain various types of antioxidants, each with different mechanisms of action. Additionally, factors such as maturity stage, agricultural practices, temperature, and environmental conditions can influence antioxidant content in fresh fruits (Souza *et al*., 2008; González-Aguilar *et al*., 2008; Agredano-De la Garza *et al*., 2021a). In this regard, the DPPH antioxidant capacity results in this study were lower than those reported by Medina-Torres *et al*. (2021) in nanche fruits, with values up to 346.6 mg AAE/100 g^{-1}, but comparable to values of 295.12 mg AAE/100 g.f.w. in a group of Brazilian nanche selections reported by Almeida *et al*. (2011). Likewise, some studies have reported varying antioxidant activities determined by the ABTS method, ranging from a minimum of 27.65 to a maximum of 331.29 mg AAE/100 g^{-1} (Medina-Torres *et al*., 2021). Moo-Huchin *et al*. (2014) also reported values between 193.22 and 195.48 mg AAE/100 g.f.w. in fruits from Yucatán, Mexico. These findings suggest that the antioxidant capacity of T1 and T2 treatments in this study falls within the average range compared to previously reported values for different nanche varieties, a characteristic worth highlighting. On the other hand, the FRAP method evaluates the total antioxidant capacity by reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. According to the FRAP method results (Figure 3K), T1 fruits reached a maximum value of 21.71 mg AAE/100 g.f.w. while T2 fruits reached 24.18 mg AAE/100 g.f.w. during storage, with statistically significant differences. On the other hand, Medina-Torres *et al*. (2021) reported FRAP-based antioxidant activities in nanche fruits ranging from 28.81 to 413.88 mg AAE/100 g^{-1}, surpassing the values obtained in this study. In conclusion, the results of this study confirm that nanche pulp possesses antioxidant capacity due to the presence of phenolic compounds, flavonoids, and carotenoids (Mariutti *et al*., 2014; Pires *et al*., 2021). Therefore, nanche fruit can be considered a food with nutraceutical potential due to its bioactive compound content (Almeida *et al*., 2024). However, a high concentration of bioactive compounds in fresh tissues (in this case, nanche pulp) does not guarantee their bioavailability upon consumption (González-*Aguilar et al*., 2008). Consequently, further studies are recommended to determine optimal storage conditions using refrigeration systems to preserve these compounds. Such studies should account for factors including environmental conditions, maturity stage, species, and agricultural practices.

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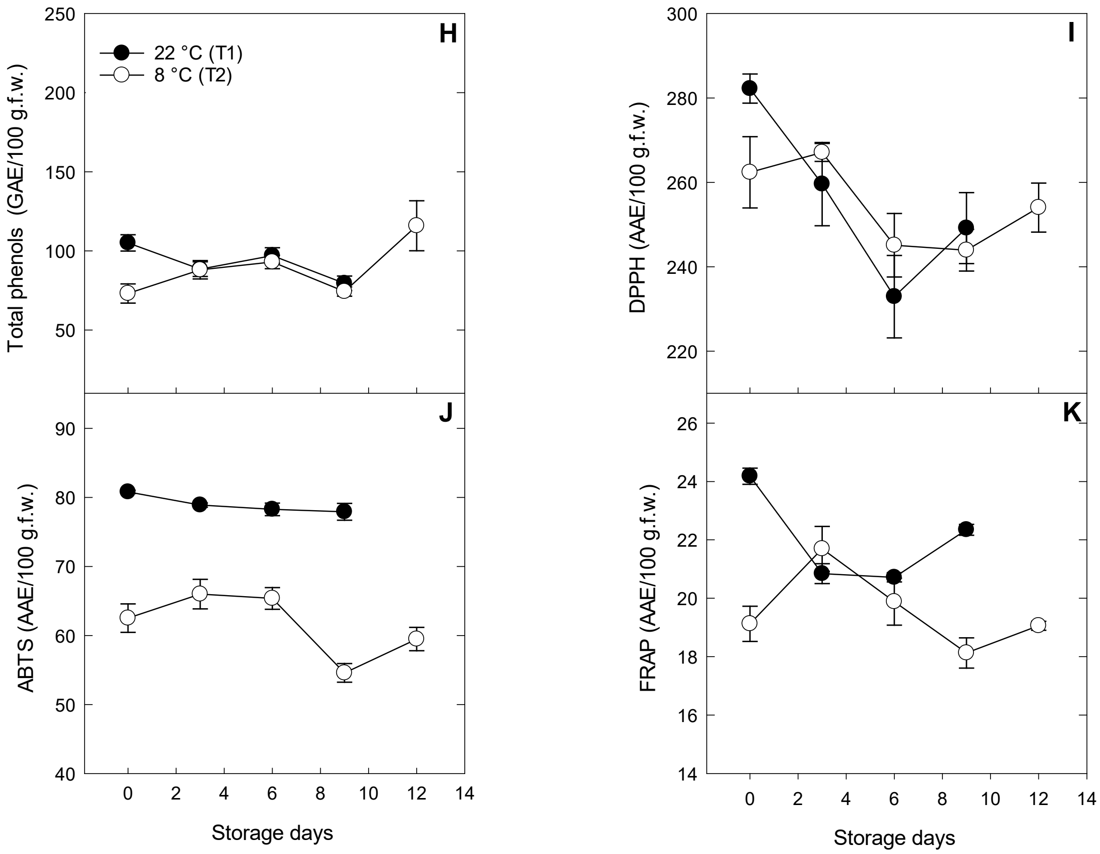

Figure 3. Total phenols (H), DPPH (I), ABTS (J) and FRAP (K), each point represents the mean of six observations and its standard error.

Conclusions

The use of cold storage systems for nanche fruit significantly reduced weight and firmness loss without altering color components compared to handling under ambient conditions. Likewise, total soluble solids (°Brix) were lower in fruits stored at 8 °C compared to those stored at 22 °C, and titratable acidity (% citric acid) remained at low levels under 8 °C storage. Furthermore, total phenolic compounds and antioxidant capacity showed values above average, indicating that refrigerated storage systems can be a feasible approach for postharvest control of nanche fruit, minimizing physicochemical changes while preserving their antioxidant potential.

Author contributions

Work conceptualization, JOJZ and CARG; Methodology development, JOJZ and RBM; Software management, PUBR; Experimental validation, CARG and PUBR; Data analysis, JOJZ, CARG, PUBR, and RBM; Data management, JOJZ and RBM; Manuscript writing and preparation, JOJZ, GGLG, and CARG; Writing, review, and editing, JOJZ and CARG; Project administration, RBM and GGLG; Funding acquisition, RBM and PUBR.

All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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