REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.13.e1905
ISSN 2007-3380

Original article / Artículo original

Susceptibility to chilling injury in five mango cultivars and its relationship with phenolics compounds and antioxidant capacity

Susceptibilidad al daño por frío en cinco cultivares de mango y su relación con los compuestos fenólicos y capacidad antioxidante

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Please cite this article as/Como citar este artículo: Vega-García, M.O., Ayón-Reyna, L.E., López Velázquez, J.G. López Angulo, G., Delgado Vargas, F., López López, M.E. (2025). Susceptibility to chilling injury in five mango cultivars and its relationship with phenolics compounds and antioxidant capacity. *Revista Bio Ciencias*, 13, e1905. https://doi.org/10.15741/revbio.13.e1905

Article Info/Información del artículo
Received/Recibido: February 11, 2025.
Accepted/Aceptado: November 14, 2025.
Available on line/Publicado: December 15, 2025.

ABSTRACT

Inadequate storage conditions lead to deterioration in mango fruit. The susceptibility to chilling injury and its relationship with phenolic compounds and antioxidant capacity were evaluated in five commercial mango cultivars (Ataulfo, Haden, Keitt, Kent, and Tommy Atkins). Fruits were stored for 21 days at chilling injury temperature (5 °C) and safe temperature (13 °C), then ripened for 7 days at 21 °C. Chilling injury susceptibility, postharvest quality, bioactive compounds, and antioxidant capacity were measured weekly. Ataulfo showed greater tolerance to chilling injury, better color retention, higher total soluble solids content, accumulation of phenolic compounds and flavonoids, but lower firmness during the ripening period at both storage temperatures. Keitt was the most susceptible, with higher firmness and lower total soluble solids content. Kent had the lowest values of bioactive compounds and antioxidant capacity, while Tommy Atkins exhibited a significant decrease in lightness and Hue angle. Results in Ataulfo indicate that the accumulation of bioactive compounds, especially flavonoids, enhances antioxidant capacity and tolerance to chilling injury, favoring its storage at low temperatures while maintaining postharvest quality.

KEY WORDS: *Mangifera indica* L., Mango cultivars, Chilling stress, Bioactive compounds, Antioxidant activity.

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ISSN 2007-3380

RESUMEN

Condiciones de almacenamiento inadecuadas ocasionan deterioro en los frutos de mango. Se evaluó la susceptibilidad al daño por frío y su relación con los compuestos fenólicos y la capacidad antioxidante en cinco cultivares comerciales de mango (Ataulfo, Haden, Keitt, Kent y Tommy Atkins). Durante 21 días se almacenaron los frutos a temperatura de daño por frío (5 °C) y temperatura sana (13 °C), posteriormente se maduraron durante 7 días a 21 °C. Se midió semanalmente la susceptibilidad al daño por frío, la calidad poscosecha, compuestos bioactivos y capacidad antioxidante. Ataulfo presentó mayor tolerancia al daño por frío, mejor conservación del color, mayor contenido de sólidos solubles totales, acumulación de compuestos fenólicos y flavonoides, pero menor firmeza durante el período de maduración en ambas temperaturas de almacenamiento. Keitt fue el más susceptible, con mayor firmeza y menor contenido de sólidos solubles totales. Kent mostró los valores más bajos de compuestos bioactivos y capacidad antioxidante, mientras Tommy Atkins evidenció una disminución significativa en luminosidad y ángulo Hue. Los resultados en Ataulfo indican que la acumulación de compuestos bioactivos, especialmente flavonoides, mejora la capacidad antioxidante y tolerancia al daño por frío, favoreciendo su almacenamiento a temperaturas bajas, manteniendo la calidad poscosecha.

PALABRAS CLAVE: *Mangifera indica* L, Cultivares de mango, Estrés por frío, Compuestos bioactivos, Actividad antioxidante.

Introduction

Mango (*Mangifera indica* L.) is an economically important tropical fruit that serves a good source of bioactive compounds that play a significant role in quenching and neutralizing free radicals, specially superoxide ion (Rathod *et al*., 2023). Mango pulp contains high levels of flavonoids (quercetin, proanthocyanidins, isoquercetin, and fisetin), phenolic acids (gallic acid, protocatechuic acid, ferulic acid, tannic acid, chlorogenic acid, and caffeic acid), and catechins (epicatechin, epigallocatechin, and epicatechin gallate). All these compounds act as natural antioxidants with the potential to protect the human body against several diseases due to their anti-inflammatory, anti-mutagenic, and anticancer activities (Chagas *et al*., 2022). In addition, some of these compounds have antibacterial and antifungal properties (Hichri *et al*., 2011).

However, mango fruit ripens and deteriorates quickly during storage at room temperature, which reduces the number of bioactive compounds and shortens its shelf life (Maldonado-Celis *et al*., 2019). The storage under refrigeration allows extending the shelf life of mango fruit, but low temperatures make it susceptible to chilling injury (CI) when exposed to temperatures below 13 ºC.

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Cold stress alters cellular membranes because induces changes in the composition of membrane lipids (conversion of liquid-crystal phase to solid-gel), which inhibits the activity of membrane-bound enzymes or transporters, increases respiration, promotes the production of ethanol and acetaldehyde and triggers the accumulation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ion (O_2^-), and hydroxyl ion (OH^-). These alterations produce symptoms such as darkening of lenticels, surface pitting, superficial scald, irregular ripening, and necrotic areas (Ghasemnezhad *et al*., 2008). The intensity and speed of this deterioration are associated with the cultivar, state of ripeness, storage temperature, and the fruit's capacity to activate the antioxidant defense system that can repair oxidative damage (López-López *et al*., 2018).

Some studies on mango fruit agree that the increase in bioactive compounds, such as flavonoids and phenolic compounds, increases the antioxidant capacity of the tissue. According to Kim *et al*. (2009) the increase in these compounds provides a protective effect due to the stabilization of cell membranes and the reduction of oxidative stress. In agreement with this, several investigations indicate that changes in these parameters during storage at low temperatures and their relationship with chilling injury susceptibility in different mango cultivars (Kananke *et al*., 2018). However, researchers have not studied this relationship in cultivars of economic importance for the state of Sinaloa, Mexico. Therefore, the aim of this study was to evaluate the relationships among phenolic compounds, antioxidant capacity, and chilling injury tolerance in five commercially important mango cultivars in Sinaloa, Mexico.

Material and Methods

Sample preparation

Five mango cultivars (Ataulfo, Haden, Keitt, Kent, and Tommy Atkins) were obtained from a local producer in Culiacan, Sinaloa, Mexico (24° 46' 23'' LN y 107° 32' 56'' LW, a 26 ma.s.l). Fruits were harvested in stage 2 (8 - 17 °Brix) and selected based on size (250 - 450 g according to cultivar), peel color uniformity, and absence of physical damage. The fruits were immediately transferred to the Postharvest Physiology and Technology Laboratory of the Postgraduate Program in Food Science and Technology-UAS (Sinaloa, México), washed, and disinfected with sodium hypochlorite (300 µL/L) for 5 min. Fruits were stored at refrigeration temperatures of 13 °C (non-chilling) and 5 °C (chilling), both at 90% relative humidity, for a total of 21 days. During this period, samples were removed every 7 days to evaluate the effect of cold storage. After the completion of the 21-day storage, the last set of fruits was transferred to 21 °C and held for 7 days to allow ripening and simulate post-storage commercial conditions.

Chilling injury index (CII)

CII was determined according to the methodology described by López-López *et al*. (2018). A total of 125 fruits were evaluated per treatment (25 fruits per storage level at 5 ºC). The criteria to evaluate the symptoms were A: lenticels darkening, B: pitting, C: blanching, D: irregular ripening, and E: necrotic areas. The severity of the symptoms was visually measured as injury level (IL)

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using a five-point scale based on the percentage of the tissue affected for each criterion (0=no tissue affected, 1=1-25 % of tissue affected, 2=26-50 %, 3=51-75%, and 4≥76 % of tissue affected). Chilling injury (CI) in each fruit was quantified by the CII according to the following equation

$$CII = \frac{(ILA+ILB+ILC+ILD+ILE)}{5}$$

Physicochemical quality

Color

For peel color evaluation, fruits were examined with a CR200 colorimeter (Minolta Co. Ltd., Osaka, Japan). Three areas along the circumference of each fruit were marked to ensure consistent color measurements at the same locations during storage at low temperatures (5 and 13 ºC) and throughout the ripening period (21+ 7 days at 21 ºC). The International Commission on Illumination (CIE) parameters L* (lightness), a* (red-green), and b* (yellow-blue) were registered.

Firmness

Firmness was conducted following the method of López-López *et al*. (2018). For each mango cheek, the peel was removed from the top and evaluated at three points, obtaining six measurements per fruit and 18 per cultivar. Chatillon penetrometer (DFE 100; AMETEK Inc., Largo, FL, USA) was used with an 11 mm diameter flat tip, operating at a constant speed of 50 mm/min and a penetration depth of 5 mm. The results are expressed in Newtons (N).

Total soluble solids

Total soluble solids (TSS) were quantified following the official AOAC method 22.014 (2012) with a manual refractometer (Atago, Fisher Scientific, GA, USA). For each sample, a drop of mango pulp juice was placed directly on the refractometer, and at least 3 readings were recorded per fruit. TSS were expressed as the percentage of sugar (ºBrix).

Analysis of bioactive compounds

Methanol extracts (ME)

One gram of freeze-dried pulp tissue was extracted under agitation with 10 mL of methanol for three days (4 °C); the solvent was replaced every 24 h. The extract was then filtered and centrifuged (12,000 g for 15 min) to eliminate any residual tissue. The solvent was removed under vacuum (40 °C) with a rotary evaporator (BÜCHI Labortechnick AG, Switzerland), which was resuspended in 4 mL of methanol and stored at -70 °C in darkness until use.

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Total phenolics

The determination of total phenolic content was undertaken using the Folin–Ciocalteu colorimetric method described by López-López *et al*. (2018). Dilution of the ME with methanol was performed considering the mango cultivar and the standard curve (Ataulfo 1:4, Haden 1:2, Kent 1:2, Keitt 1:1, and Tommy Atkins 1:1). A 20 µL aliquot of the dilution were oxidized with 180 µL of the Folin-Ciocalteu reagent (1:8, v/v) plus 50 µL of Na_2CO_3 (7 %, w/v) for 90 min, followed by measurement of the absorbance at 750 nm using a Microplate Reader (Synergy TM HT Multi-Detection, Biotek, Inc., Winooski). A standard curve was prepared using gallic acid (50-500 µg/mL). Total phenolics concentration is quantified in milligrams of gallic acid equivalents (mg GAE) per 100 grams of fresh weight (g FW).

Total flavonoids

Total flavonoids were obtained by taking a 0.5 mL aliquot of the ME, mixing it with 2 mL of deionized water, and the mixture was equilibrated with 150 µL of 5 % $NaNO_2$ for 5 min. After equilibrium, 150 µL of 10 % $AlCl_3$ (methanolic solution) was added; the mixture was allowed to stand for 1 min, and then 1 mL of 1 M NaOH was added. Finally, the volume was brought to 5 mL with deionized water, shaken, and the absorbance was read at 415 nm using a UV-VIS spectrophotometer (UNICO SQ 2800 NJ, USA). The total flavonoid content was determined using a standard curve of quercetin (10-120 mg/L) and expressed as mg quercetin equivalents (QE)/100 g FW, according to Moo-Huchin *et al*. (2014) with some modifications.

Antioxidant capacity

DPPH (2,2′-diphenyl-1-picrylhydrazyl) method

The DPPH assay was performed as described by López-Angulo *et al*. (2019) with slight modifications. A 20 µL aliquot of the ME (different dilution for each cultivar) were mixed with 180 µL of 150 mM DPPH solution in methanol. The mixture underwent incubation for 30 min at 27 °C in darkness before measuring the absorbance at 525 nm (SynergyTM HT Multi-Detection, Biotek, Inc., Winooski, VT). Trolox (Sigma-Aldrich-238813) functioned as the standard to create a curve ranging from 0 to 225 µg/mL. Activity is expressed as µmoles Trolox Equivalents (TE) (µmol TE/100 g FW).

ABTS (2,2′-Azinobis-3-ethylbenzotiazoline-6-sulfonic acid) method

The ABTS stock solution was prepared according to López-Angulo *et al*. (2019) by mixing 5 mL of 7 mM ABTS reagent with 88 µL of potassium persulfate (140 mM) and incubating it in darkness at 25 ºC for 12-16 h. The ABTS solution was combined with 7 mM phosphate-buffer solution (PBS) (pH 7.4) to reach an absorbance of 0.75 ± 0.02 at 734 nm. In assay tubes, 0.1 mL of ME diluted in methanol was mixed with 2 mL of ABTS solution and maintained in darkness for 30 min at 27 °C before measuring its absorbance at 734 nm. A Trolox calibration curve was prepared in the range of 0-225 µg/mL, and activity is expressed as µmol TE/100 g FW.

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ORAC (oxygen radical absorbance capacity) method

The ORAC assay was implemented as previously described by López-Angulo *et al*. (2019) with some modifications. 25 µL aliquot of each diluted ME was placed into a 96-well plate (black microplate). The microplate was positioned into the plate holder of the reader (SynergyTM HT Multi-Detection, BioTek, Inc., Winooski, VT), where 175 µL of fluorescein (0.96 µM) was dispensed, mixed, and incubated at 37 °C for 30 min before adding 50 µL of APPH (200 mM). The fluorescence intensity (485 nm (ex)/ 525 nm (em)) was measured for 60 min at 37 °C with 2 min intervals. The blank and Trolox standard curve (25–125 µM) were analyzed in the same way as the sample. The antioxidant capacity was determined by contrasting the areas under the curves obtained with Trolox and the samples. Results were expressed as µmol TE/ 100 g FW.

Statistical analysis

Data were analyzed by a two-way analysis of variance; the factors were cultivars (Ataulfo, Haden, Keitt, Kent, Tommy Atkins) and days of storage at 5 or 13 ºC (0, 7, 14, 21, 21+7). Significant differences (95% confidence level) between means were determined by applying Fisher's least significant difference test. Pearson correlation coefficients (r) between data sets were evaluated at a 90% confidence level. All analyses were performed using the software Statgraphics Plus 5.1 (Statistical Graphics, Rockville, MD).

Results and Discussion

Chilling injury index (CII)

Figure 1 shows the susceptibility of different mango cultivars at low temperatures. CII of the Ataulfo cultivar increased during the first 14 days of storage, after which it remained constant, even during ripening storage, making it the cultivar with the lowest symptom development (Figure 1A). On the other hand, the Keitt cultivar showed the highest CII, with symptoms such as lenticel darkening, pitting, and necrotic areas, especially after transferring the fruits to 21 ºC (Figure 1B). Haden, Kent, and Tommy Atkins cultivars exhibited similar behavior, without differences among them at both temperatures (5 and 21 ºC).

Cellular diffusion resulting from membrane destabilization (including lipid oxidation, increased membrane permeability, and elevated activity of lipoxygenase and phospholipase enzymes), along with cytoplasm disruption and chloroplast swelling, explains the intensity of the symptoms (Aghdam *et al*., 2013). Additionally, reductions in amylase activity, suppression of ethylene biosynthesis and starch degradation (Nair & Singh, 2003), as well as decreases in bioactive compounds and antioxidant capacity that limit the fruit´s defense mechanism (Abidi *et al*., 2014).

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Ataulfo has several characteristics that differ from the other cultivars, such as high levels of soluble sugars and phenolic compounds (Manthey & Perkins, 2009). So, this difference could reflect the higher chilling tolerance of this variety because soluble sugars function as osmoprotectors and provided stability to plasma membrane proteins (Singh & Singh, 2012), while phenolic compounds decrease oxidative stress by neutralizing the free radicals produced during storage at low temperature (Palafox-Carlos *et al*., 2012). Researchers previously observed low CI susceptibility (<50% surface affected) in other cultivars such as Choke Anan stored at 6 °C (Kondo *et al*., 2005), and Zill stored at 5 °C (Li *et al*., 2012).

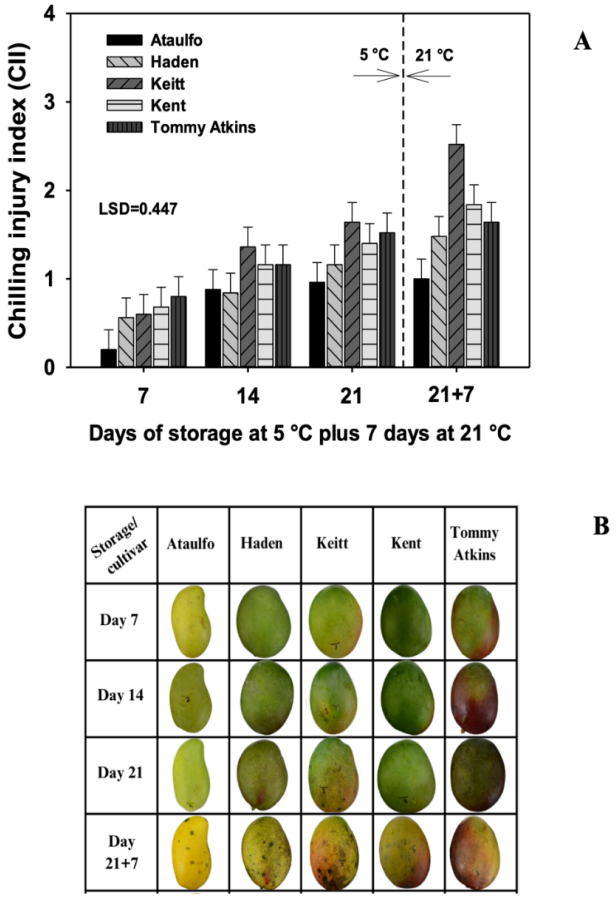

Figure 1. Chilling injury index (CII) (A) and visible symptoms of CI (B) of five mango cultivars (Ataulfo, Haden, Keitt, Kent, and Tommy Atkins) stored at 5 ºC plus 7 days at 21 ºC.

Vertical bars on columns represent minimal significant difference of the means of five replicates.

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Color

Ataulfo, Haden, and Kent did not present significant changes in Lightness during storage at 5 ºC, while Keitt and Tommy Atkins showed a reduction of L* (Figure 2A). Ataulfo and Tommy Atkins presented the highest and the lowest L* values, respectively, with a significant difference between them. During storage at 13 ºC, Ataulfo and Haden showed constant L* values, while Keitt and Kent showed a slight diminution, and Tommy Atkins presented a greater decrease in this parameter (Figure 2B). During ripening (21+7 days, 5 and 13 ºC), Keitt and Tommy Atkins did not have changes in L*, whereas the rest of the cultivars presented an increase. Ataulfo cultivar had the highest L* values throughout storage due to chlorophyll degradation and carotenoid synthesis. On the other hand, the decrease in luminosity may result from the loss of brightness and tissue darkening caused by CI symptoms such as lenticel darkening, irregular ripening, and, in some cases, necrotic areas (Figure 1B).

Tommy Atkins had the lowest Hue angle (Hueº) values throughout storage, regardless of the storage temperature. These values correlated well with the red hue (presence of anthocyanins) and showed a significant difference ($p < 0.05$) compared with the other cultivars (Figure 2C, 2D). During storage at 5 ºC, only Haden maintained the same values, while the rest of the cultivars showed a slight reduction (Figure 2C) due to cold stress. In storage at 13 ºC, Ataulfo, Haden, Kent, and Tommy Atkins exhibited a reduction in the Hue angle (Figure 2D) and showed a higher proportion of yellow peel than during normal ripening (Figure 1B).

Previous studies obtained similar results in Kensington mango (Zahara & Singh, 2011), Ataulfo (Robles-Sánchez *et al*., 2009; Palafox-Carlos *et al*., 2012), and Nam Dok Mai (Junmatong *et al*., 2015) stored at chilling temperatures and during ripening. These changes are related to pigment degradation and synthesis, as well as variations in parameters a* and b*, which favor green-yellow coloration at the end of storage at low temperatures and a yellow coloration during ripening. Palafox-Carlos *et al*. (2012) observed that a high Hue° value in mango fruit corresponds to a small portion (<20% fruit surface) of yellow color, whereas an increase in this portion (>21% fruit surface) leads to a considerable decrease in Hueº and a change in fruit coloration. This behavior results from carotenoid biosynthesis triggered by the climacteric rise in the fruit, initiated by ethylene action.

Firmness

During the first 7 days of storage at 5 ºC, all mango cultivars except Tommy Atkins, presented firmness reduction (Figure 2E). On the rest of the storage. Ataulfo and Keitt remained unchanged, while Kent and Haden showed a reduction. and Tommy Atkins showed an increase without a statistical difference among them. During ripening (21 °C) after chilling storage, all cultivars showed a significant reduction in firmness, obtaining similar values ($p > 0.05$). During storage at 13 °C. all cultivars presented a significant reduction of firmness at day 7, Ataulfo and Kent showed the greatest loss of firmness (76.89% and 68.78%, respectively) (Figure 2F). From 14 to 21 days; Haden, Kent and Tommy Atkins showed a significant reduction while Ataulfo and

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Keitt did no present significant changes. Keitt cultivar resulted with the highest values at the end of 13 °C storage (96.04 N).

After 7 days of ripening at 21 °C (21+7 days), the different cultivars exhibited a significant loss of firmness, Ataulfo and Keitt presented the least and greatest loss (15.72% and 90.88%, respectively) compared to day 21 (13 ºC). It is important to mention that the values were lower than the commercial standard accepted for fresh mango (20 N) according to Romero–Gomezcaña *et al*. (2006). Dea *et al*. (2010) related the firmness loss in mango to a decrease in the fluidity of membrane micro-domains and to damage to proteins that together lead to cell membrane rigidity during CI, especially during the transfer of fruit to ripening conditions. Sayyari *et al*. (2011) related this reduction in firmness to the loss of cell wall integrity, since a breakdown of pectic substances occurs, and increases pectin solubility. On the other hand, increased firmness retention during storage at chilling temperatures may result from the loss of the fruit's ability to convert 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene during storage, thereby limiting normal fruit ripening (Lederman *et al*., 1997).

Previous investigations reported similar results in Kensington (Nair & Singh, 2003), and Carabao (Cantre *et al*., 2014) during storage at chilling temperatures, as the fruit showed a reduction in firmness during the first days of storage. On the other hand, in the cultivars Irwin (Shivashankara *et al*., 2004) and Nam Dok Mai (Junmatong *et al*., 2015) retained firmness during storage at 5 °C and subsequent storage at room temperature. This retention was due to irregular fruit ripening, which increased cell rigidity.

Total soluble solids (TSS)

TSS relates to fruit ripeness, and an increase in this parameter indicates greater sweetness. In this study, the Ataulfo cultivar had the highest SST values across storage temperatures, with a statistical difference ($p < 0.05$) compared with the other cultivars (Figures 2G, 2H). Greater hydrolysis of starch and synthesis of simple sugars explain this behavior. However, when comparing storage temperatures, all cultivars show higher TSS content at 13 ºC (Figures 2G and 2H). On day 21, the Keitt cultivar showed the lowest TSS content at 5 °C (8.2 °Brix), but a higher value (12.73 ºBrix) at 13 °C. A lower storage temperature reduces the respiration rate and therefore decreases the conversion of starch into sugars (López-López *et al*., 2018). After storage at 5 °C, all cultivars increased their TSS during ripening. At 13 °C, Keitt and Tommy Atkins increased their TSS due to starch degradation into simple sugars. Ataulfo and Kent maintained constant levels due to a balance between sugar consumption and production, and Haden showed a reduction associated with fruit senescence and the consequent energy expenditure. Montalvo *et al*. (2007) reported that fruit with higher TSS accumulate more simple sugars, which function as osmoregulators in the cell and reduce its permeability, allowing greater tolerance to storage at low temperatures.

The TSS content in the Ataulfo cultivar could contribute to CI tolerance, consistent with the CII results, where Ataulfo developed the fewest symptoms. Minimal changes in TSS indicate that low temperatures damage metabolism by limiting the activity of hydrolytic enzymes needed to

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produce simple sugars (Siddiq *et al*., 2013; Junmatong *et al*., 2015). Ding *et al*. (2007) mentioned that mango fruit stored at 5 °C synthesized less TSS than fruit stored at 14 °C.

Bioactive compounds

Haden, Keitt, Kent, and Tommy Atkins cultivars showed similar values throughout storage at 5 °C, averaging 25 mg gallic acid/100 g FW (Figure 3A). On the other hand, Ataulfo showed the highest phenolic content at the beginning of the storage with 52.5 mg gallic acid/100 g FW, and by the end of the storage at 21 °C, fruit had a phenolic content of 109.9 mg gallic acid/100 g FW. Fruits transferred to 21 °C showed a reduction in total phenolics, which was most significant in Ataulfo. Nevertheless, this cultivar still retained the highest phenolic content. The five cultivars stored at 13 ºC showed similar results, except for Kent, which increased phenolics by 33.6% on day 21+7 (Figure 3B).

On day 0, Tommy Atkins had the highest flavonoid content (Figure 3C). However, only Ataulfo showed a significant increase during storage at 5 °C. The other cultivars showed no significant changes in total flavonoids except for Kent, which decreased after 14 days of storage. None of the cultivars showed significant changes during the ripening period. During storage at 13 ºC, Ataulfo significantly increased flavonoid synthesis, reaching 116 mg Quercetin/100 g FW at day 21, which is 30.7% higher than the value obtained at 5 ºC (Figure 3D). The other cultivars maintained flavonoid content near 37 mg Quercetin/100 g FW. During storage at 21 °C, Ataulfo increased its flavonoid content significantly, reaching an average value 3.93-fold higher than those of the other cultivars.

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ISSN 2007-3380

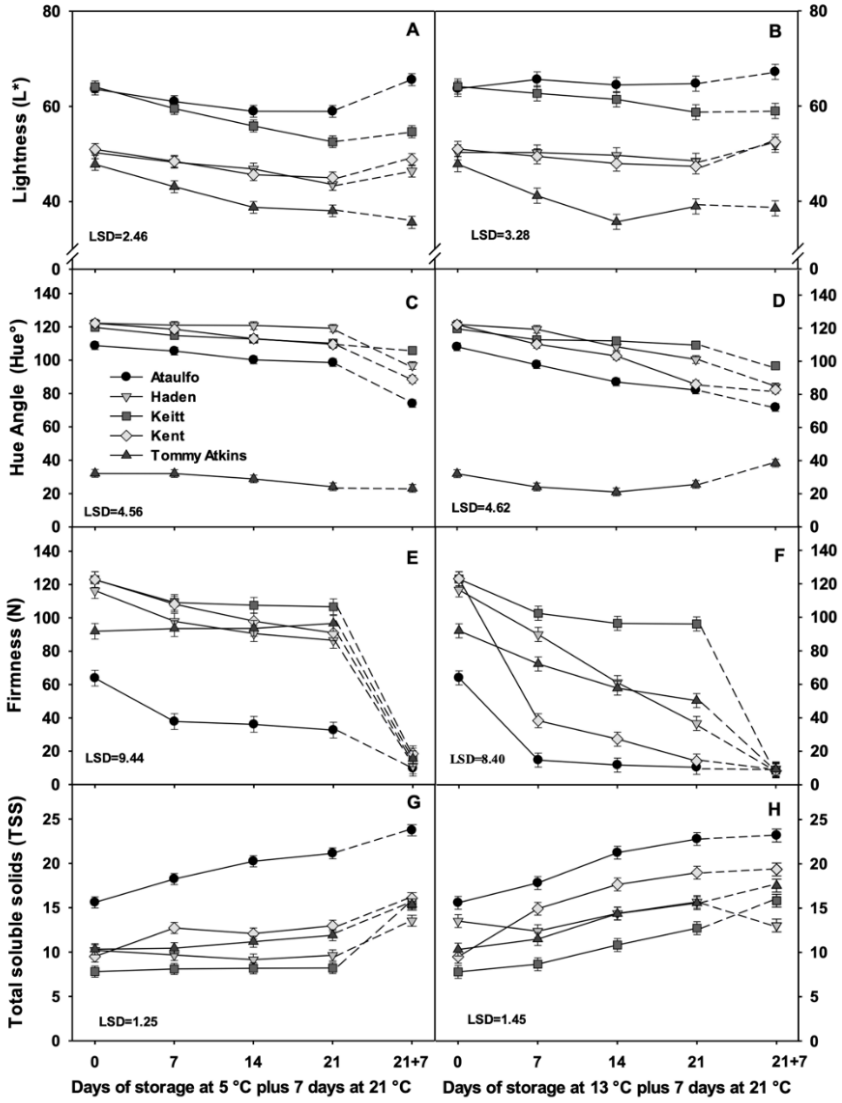

Figure 2. Parameters of Lightness (A, B), Hue angle (C, D), Firmness (E, F), and Total soluble solids (G, H) of five mango cultivars (Ataulfo, Haden, Keitt, Kent, and Tommy Atkins) stored at 5 ºC and 13 ºC plus 7 days at 21 ºC.

Data are the mean of nine replicates; vertical bars represent significant differences among treatments ($p < 0.05$) based on the least significant differences by Fisher's test.

The cellular mechanisms that prevent cold stress symptoms generally maintain membrane and cell wall integrity, sustain higher-energy status, and enhance the antioxidant system (Gao

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et al., 2016), which induces the accumulation of bioactive compounds. This corresponds with the highest accumulation of flavonoids and total phenolics in Ataulfo during storage at 5 and 13 °C. The increase in bioactive compounds in this cultivar likely stabilizes internal membranes, cell walls and the extracellular space, limiting free radical diffusion and reducing susceptibility to low temperature. Fruits biosynthesize these compounds via the shikimic acid pathway from precursors derived from carbohydrate metabolism, such as glycolysis (Tomás-Barberán & Espín, 2001). Sugars convert to acetyl-CoA and then to malonyl-CoA (polyacetate pathway), which, combined with p-coumaryl-CoA (shikimic acid pathway and tyrosine) (Hichri *et al*., 2011) and the key enzyme phenylalanine ammonia-lyase (PAL), favors the synthesis and accumulation of phenolic compounds, flavonoids and quinones (Tomás-Barberán & Espín, 2001). Ataulfo´s highest accumulation of bioactive compounds and soluble sugars during ripening contributed to reducing its CII.

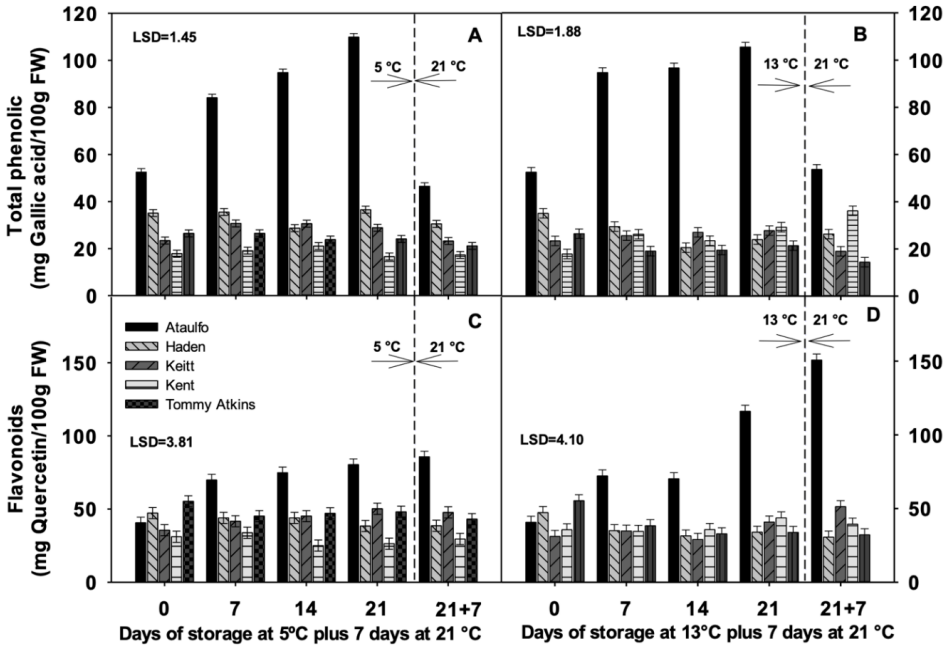

Figure 3. Content of total phenolics (A, B) and Flavonoids (C, D) of five mango cultivars (Ataulfo, Haden, Keitt, Kent, and Tommy Atkins) stored at 5 ºC and 13 ºC plus 7 days at 21 ºC.

Data are the mean of nine replicates; vertical bars represent significant differences among treatments ($p < 0.05$) based on the least significant differences by Fisher's test.

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Robles-Sánchez *et al*. (2009) found that phenolics and flavonoids reduce deteriorative reactions through their redox properties, acting as hydrogen donors, singlet-oxygen quenchers, and peroxide decomposers. Our results agree with previous findings in avocado (Sivankalyani *et al*., 2015) stored at low temperatures, where the fruit increased bioactive compounds, which was associated with less susceptibility to CI. However, other studies on mango cultivars such as Wacheng (Zhao *et al*., 2006), Ataulfo (Robles-Sánchez *et al*., 2009) and Choke Anan (Kondo *et al*., 2005) showed decreases in total phenolics and pulp browning, attributing these changes to oxidation of phenolic compounds by polyphenol oxidase enzyme activity (Robles-Sánchez *et al*., 2009). Additionally, Siddiq *et al*. (2013) and Cantre *et al*. (2014) found that Tommy Atkins and Carabao cultivars increased phenolic content during the first day of low-temperature storage, thereby enhancing their antioxidant capacity; however, our results show that Tommy Atkins decreased total phenolics. These findings suggest that the synthesis and accumulation of phenolics and flavonoids enhance fruit tissue tolerance to chilling stress.

Antioxidant capacity

During storage at 5 ºC, Ataulfo exhibited the highest antioxidant capacity, as measured by the DPPH method, with 6.1 times that of the other cultivars on day 21 (Figure 4A). At 21 °C, all cultivars decreased their antioxidant capacity; Ataulfo maintained the highest values, while the others did not differ significantly. Ataulfo increased its antioxidant capacity during storage at 13 °C compared to 5 ºC, reaching the highest values after 21 days (Figure 4B). On the other hand, Haden showed the lowest value, which was 10-fold lower than Ataulfo´s. The remaining cultivars showed no significant differences in antioxidant capacity. Storage at 21 ºC reduced Ataulfo's antioxidant capacity by a factor of 2.06, while Haden, Keitt, and Kent maintained constant levels.

In the ABTS assay, antioxidant capacity ranged from 203.3 to 1088.6 µmol TE/100 g FW during storage at 5 ºC (Figure 4C). Ataulfo reached the highest values after 21 days, followed by Tommy Atkins, Haden, Keitt, and Kent. During storage at 21 °C, Haden and Tommy Atkins exhibited the highest activity, with values 3.31 times higher than those of the other cultivars (Figure 4D). At 21 °C, cultivars did not present significant changes except Keitt, which significantly reduced antioxidant capacity.

During storage at 5 °C, Tommy Atkins showed the highest antioxidant capacity measured by the ORAC assay, with values ranging from 439.9 to 619.9 µmol TE/100 g FW (Figure 4E); while Haden had the lowest value. After 7 days at 21 °C, Ataulfo, Haden, and Kent increased their activity, and Tommy Atkins maintained the highest antioxidant capacity. When stored at 13 ºC, all cultivars increased their antioxidant capacity (Figure 4F); Ataulfo reached the highest activity at day 21, a value 2.26-fold higher than the other cultivars. Similarly, Haden had the lowest value, significantly different ($p < 0.05$) from the other cultivars. After ripening storage, Ataulfo increased its antioxidant capacity by 1.42-fold compared to its previous storage point, while Keitt significantly reduced it; the rest of the cultivars did not show significant changes.

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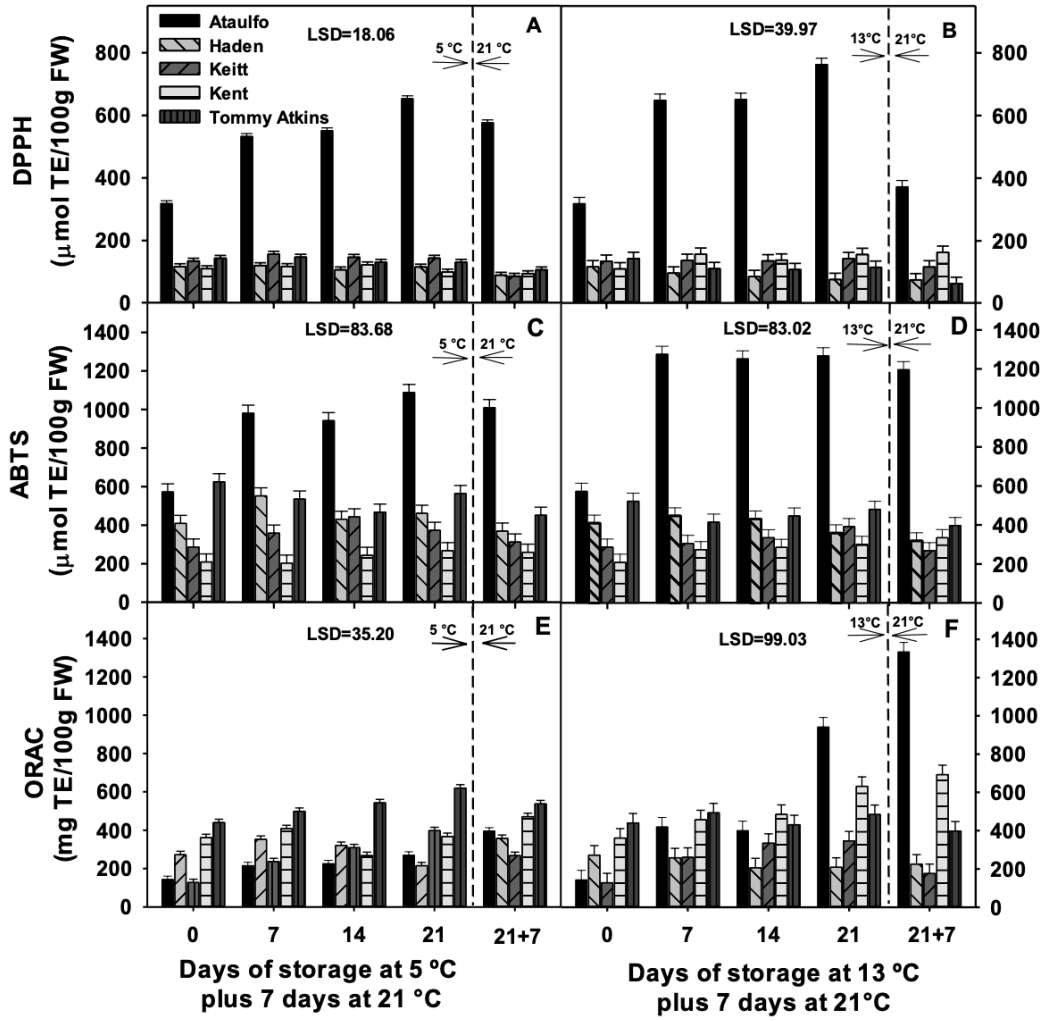

Figure 4. Antioxidant capacity by DPPH (A, B), ABTS (C, D), and ORAC (E, F) of five mango cultivars (Ataulfo, Haden, Keitt, Kent, and Tommy Atkins) during storage at 5 ºC and 13 ºC.

Data are the mean of nine replicates; vertical bars represent significant differences among treatments ($p < 0.05$) based on the least significant differences by Fisher's test.

The antioxidant capacity showed different correlations with total phenolics and total flavonoids depending on the cultivar, time, and storage temperature. The accumulation of total phenolics or flavonoids seems to increase antioxidant capacity and reduce mango fruit susceptibility to low temperature. Robles-Sánchez *et al*. (2009) linked antioxidant capacity to the accumulation of phenolic compounds such as gallic, protocatechuic, chlorogenic, ferulic, and caffeic acids, which

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exhibit high reducing power. Noratto *et al*. (2010) studied different mango cultivars and found that Ataulfo had the highest antioxidant capacity (ORAC) with a value of 327 µmol TE/100 g, which is lower than the values obtained in the present study. They attributed this difference to the extraction method, which produced phenolic extracts lacking ascorbic acid, soluble proteins, and reducing sugars that may enhance antioxidant capacity in fruit.

We found a strong correlation between the presence of CI symptoms and the decrease in antioxidant capacity; both linked to a reduction in total phenolics and flavonoids. Pearson's analysis showed that total phenolic correlated with ABTS for Keitt (r=0.846 $p < 0.071$), and DPPH for Kent and Tommy Atkins (r=0.894 $p < 0.041$; r=0.989 $p < 0.001$). The three methods correlated with flavonoids in Ataulfo (r=0.946, 0.952, 0.839 $p < 0.015$, 0.013, 0.075, respectively), with ORAC in Keitt (r=0.934 $p < 0.020$), and with ABTS in Tommy Atkins (r=0.852 $p < 0.067$). (Table 1). Likewise, Razzaq *et al*. (2014) reported a significant reduction in antioxidant capacity during mango ripening, as senescence and oxidative stress lead to an excess of free radicals and a decrease in antioxidant compounds. Palafox-Carlos *et al*. (2012) suggested that fruit ripening increases cellular activity and respiration, promoting the reduction of bioactive compounds used to neutralize free radicals generated at the end of the electron transport chain. On the other hand, Shivashankara *et al*. (2004) indicated that antioxidant capacity was not related to total phenol, quercetin, or β-carotene content in the Irwin mango. The reduction of these compounds and antioxidant activity could increase susceptibility to chilling injury.

Table 1. Pearson's correlation between bioactive compounds and antioxidant capacity of five mango cultivars stored at 5 ºC for 7 days at 21 ºC.

Cultivar	Bioactive compounds	ABTS	DPPH	ORAC
Ataulfo	Phenolics	0.603	0.555	-0.182
	Flavonoids	0.946**	0.952**	0.839*
Haden	Phenolics	0.739	0.534	-0.56
	Flavonoids	0.524	0.185	0.111
Keitt	Phenolics	0.788	0.846*	0.52
	Flavonoids	-0.254	0.455	0.934**
Kent	Phenolics	0.894**	-0.341	-0.666
	Flavonoids	0.033	-0.758	0.599
Tommy Atkins	Phenolics	0.989**	0.772	-0.531
	Flavonoids	0.561	0.852*	-0.496

Note. *p* values are presented with symbol *. (no symbol) Non-significant correlation; (*) significant correlation with $p < 0.10$; (**) significant correlation with $p < 0.05$.

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ISSN 2007-3380

Conclusion

The five mango cultivars showed different susceptibility to chilling injury (CI), with Keitt being the most susceptible and Ataulfo the least. Greater accumulation of flavonoids and higher antioxidant capacity contributed to Ataulfo's resistance to CI. These results suggest that producers can store Ataulfo at 5 °C to extend its shelf life during marketing. This cultivar is highly appreciated in Mexico and is a valuable source of bioactive compounds in the diet.

Author contributions

Conceptualization of the work, MOVG, MELL; methodology development, MOVG, LEAR, JGLV; software management, MELL; experimental validation, MELL, MOVG; results analysis, MOVG, LEAR, MELL; data management, MOVG, LEAR, MELL; writing and manuscript preparation, MOVG, LEAR, MELL; drafting, review, and editing, MELL, JGLV, GLA, FDV; project administration, MELL.

All authors of this manuscript have read and approved the published version.

Funding

This research did not receive external funding.

Ethical Statements

Not applicable.

Informed Consent Statement

Not applicable.

Acknowledgments

We sincerely appreciate the invaluable support provided by the Postharvest Physiology and Technology Laboratories of FCQB-UAS and the Natural Products Laboratory of FCQ-UAS, whose assistance was essential for the completion of this research project.

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ISSN 2007-3380

Conflict of Interest

"The authors declare no conflict of interest."

References

Abidi, W., Cantín, C. M., Jiménez, S., Giménez, R., Moreno, M. A., & Gogorcena, Y. (2014). Influence of antioxidant compounds, total sugars and genetic background on the chilling injury susceptibility of a non-melting peach (Prunus persica (L.) Batsch) progeny. *Journal of the Science of Food and Agriculture*, 95(2), 351–8. https://doi.org/10.1002/jsfa.6727

Aghdam, M. S., Sevillano, L., Flores, F. B., & Bodbodak, S. (2013). Heat shock proteins as biochemical markers for postharvest chilling stress in fruits and vegetables. Scientia Horticulturae, 160, 54–64. https://doi.org/10.1016/j.scienta.2013.05.020

AOAC [Association of Official Analytical Chemists]. (2012) Official Methods of Analysis. 16th ed. S William (ed). Published by the Association of Official Analytical Chemists. Washington, D.C. USA. CD-Rom. https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf

Cantre, D., Herremans, E., Verboven, P., & Nicolaï, B. M. (2014). Characterization of the 3-D cellular structure of mango (Mangifera indica L. cv. Carabao) during chilling injury development using X-ray computed microtomography. *Inside Food Symposium*, 9–12. https://doi.org/10.1016/j.ifset.2013.12.008

Chagas, M. S. Behrens, M. D. Moragas-Tellis, C. J., Penedo, X. M, Silva, A. R., & Gonçalves-de-Albuquerque, G. F. (2022). Flavonols and Flavones as Potential anti-Inflammatory, Antioxidant, and Antibacterial Compounds. *Oxidative Medicine and Cellular Longevity*, 2022 (1), 9966750 https://doi.org/10.1155/2022/9966750

Dea, S., Brecht, J. K., Nunes, M. C., & Baldwin, E. A. (2010). Occurrence of Chilling Injury in Fresh-Cut 'Kent' Mangoes. *Postharvest Biology and Technology*, 57(1), 61–71. https://doi.org/10.1016/j.postharvbio.2010.02.005

Ding, Z. S., Tian, S. P., Zheng, X. L., Zhou, Z. W., & Xu, Y. (2007). Responses of reactive oxygen metabolism and quality in mango fruit to exogenous oxalic acid or salicylic acid under chilling temperature stress. *Physiologia Plantarum*, 130(2), 112–121. https://doi.org/10.1111/j.1399-3054.2007.00893.x

Gao, H. Zhang, Z., Lv, X., Cheng, N., Peng, B. X., & Cao, W. (2016). Effect of 24-epibrassinolide on chilling injury of peach fruit in relation to phenolic and proline metabolisms. *Postharvest Biology and Technology*, 111, 390–397. https://doi.org/10.1016/j.postharvbio.2015.07.031

Ghasemnezhad, M., Marsh, K., Shilton, R., Babalar, M., & Woolf, A. (2008). Effect of hot water treatments on chilling injury and heat damage in "satsuma" mandarins: Antioxidant enzymes and vacuolar ATPase, and pyrophosphatase. *Postharvest Biology and Technology*, 48(3), 364–371. https://doi.org/10.1016/j.postharvbio.2007.09.014

Hichri, I., Barrieu, F., Bogs, J., Kappel, C., Delrot, S., & Lauvergeat, V. (2011). Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. *Journal of Experimental Botany*, 62(8), 2465–2483. https://doi.org/10.1093/jxb/erq442

Junmatong, C., Faiyue, B., Rotarayanont, S., Uthaibutra, J., Boonyakiatc D., & Saengnil K.

Revista Bio ciencias
ISSN 2007-3380

(2015). Cold storage in salicylic acid increases enzymatic and non-enzymatic antioxidants of Nam Dok Mai No. 4 mango fruit, *ScienceAsia*, 41, 12–21. doi: https://doi.org/10.2306/scienceasia1513-1874.2015.41.012

Kananke, M. D., Singh, Z., & Johnson, S. K. (2018). Cold storage temperatures and durations affect the concentrations of lupeol, mangiferin, phenolic acids and other health-promoting compounds in the pulp and peel of ripe mango fruit. *Postharvest Biology and Technology*, 139, 91-98. https://doi.org/10.1016/j.postharvbio.2017.12.003

Kim, Y., Lounds-Singleton, A. J., & Talcott, S. T. (2009). Antioxidant phytochemical and quality changes associated with hot water immersion treatment of mangoes (Mangifera indica L.). *Food Chemistry*, 115(3), 989–993. https://doi.org/10.1016/j.foodchem.2009.01.019

Kondo, S., Kittikorn, M., & Kanlayanarat, S. (2005). Preharvest antioxidant activities of tropical fruit and the effect of low temperature storage on antioxidants and jasmonates. *Postharvest Biology and Technology,* 36(3), 309–318. https://doi.org/10.1016/j.postharvbio.2005.02.003

Lederman, I. E., Zauberman, G., Weksler, A., Ilana, R., & Fuchs, Y. (1997). Ethylene-forming capacity during cold storage and chilling injury development in “Keitt” mango fruit. *Postharvest Biology and Technology*, 10 (1), 107–112. https://doi.org/10.1016/S0925-5214(96)00060-9

Li, B., Zhang, C., Cao, B., Qin, G., Wang, W., & Tian, S. (2012). Brassinolide enhances cold stress tolerance of fruit by regulating plasma membrane proteins and lipids. *Amino Acids*, 43(6), 2469–2480. https://doi.org/10.1007/s00726-012-1327-6

López-Angulo, G., Montes-Avila, J., Díaz-Camacho, S. P., Vega-Aviña, R., Ahumada-Santos, Y. P., & Delgado-Vargas, F. (2019). Chemical composition and antioxidant, α-glucosidase inhibitory and antibacterial activities of three Echeveria D C. species from Mexico. *Arabian Journal of Chemistry*, 12(8), 1964-1973. https://doi.org/10.1016/j.arabjc.2014.11.050

López-López, M. E., López-Valenzuela, J. A., Delgado-Vargas, F., López- Angulo, G., Carrillo-López, A., Ayón-Reyna, L. E., & Vega-García, M. O. (2018). A treatment combining hot water with calcium lactate improves the chilling injury tolerance of mango fruit. *HortScience,* 53(2), 217–223. https://doi.org/10.21273/HORTSCI12575-17

Maldonado-Celis, M. E., Yahia, E. M. Bedoya, R., Landázuri, P., Loango, N., Aguillón, J., Restrepo, B., & Guerrero-Ospina, J. C. (2019). Chemical composition of mango (Mangifera indica L.) fruit: nutritional and phytochemical compounds. *Frontiers in Plant Science*, 10(1073) https://doi.org/10.3389/fpls.2019.01073

Manthey, J. A., & Perkinsveazie, P. (2009). Levels of β-carotene, ascorbic acid, and total phenols in the pulp of five commercial varieties of mango (Mangifera indica L.). *Proceedings of the Florida State Horticultural Society*, 122, 303–307. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20183248110#core-collateral-purchase-access

Moo-Huchin, V. M., Estrada-Mota, I., Estrada-León, R., Cuevas-Glory, L., Ortiz-Vázquez, E., Vargas y Vargas, M. D., & Sauri-Duch, E. (2014). Determination of some physicochemical characteristics, bioactive compounds and antioxidant activity of tropical fruits from Yucatan, Mexico. *Food Chemistry*, 152, 508–515. https://doi.org/10.1016/j.foodchem.2013.12.013

Montalvo, E., García, H. S., Tovar, B., & Mata, M. (2007). Application of exogenous ethylene on postharvest ripening of refrigerated ‘Ataulfo’ mangoes. LWT - *Food Science and Technology*, 40(8), 1466-1472. https://doi.org/10.1016/j.lwt.2006.03.014

Nair, S., & Singh, Z. (2003). Pre-storage ethrel dip reduces chilling injury , enhances respiration rate , ethylene production and improves fruit quality of “Kensington” mango, Journal of Food

Agriculture and Environment, 1(2), 93-97. https://hdl.handle.net/20.500.11937/38466

Noratto, G. D., Bertoldi, M. C., Krenek, K., Talcott, S. T., Stringheta, P. C., & Mertens-Talcott, S. U. (2010). Anticarcinogenic effects of polyphenolics from mango (Mangifera indica) varieties. *Journal of Agricultural and Food Chemistry*, 58(7), 4104–4112. https://doi.org/10.1021/jf903161g

Palafox-Carlos, H., Yahia, E., Islas-osuna, M. A., Gutierrez-Martinez, P., Robles-Sánchez, M., & González, E.A. (2012). Effect of ripeness stage of mango fruit (Mangifera indica L., cv. Ataulfo) on physiological parameters and antioxidant activity. *Scientia Horticulturae*, 135, 7–13.

Rathod, N. B., Elabed, N., Punia, S., Ozogul, F., Kim, S. K., & Rocha, J. M. (2023). Recent developments in polyphenol applications on human health: A review with current knowledge. *Plants*, 12(16),1217. https://doi.org/10.3390/plants12061217

Razzaq, K., Khan, A. S., Malik, A. U., Shahid, M., & Ullah, S. (2014). Role of putrescine in regulating fruit softening and antioxidative enzyme systems in “Samar Bahisht Chaunsa” mango. *Postharvest Biology and Technology*, 96, 23–32. https://doi.org/10.1016/j.postharvbio.2014.05.003

Robles-Sánchez, R. M., Islas-Osuna, M., Astiazarán-García, H., Vázquez-Ortiz, F., Martín-Belloso, O., Gorinstein, S., & González-Aguilar, G. (2009). Quality index, consumer acceptability, bioactive compounds, and antioxidant activity of fresh-cut “ataulfo” mangoes (Mangifera indica L.) as affected by low-temperature storage. *Journal of Food Science*, 74(3), S126-S134. https://doi.org/10.1111/j.1750-3841.2009.01104.x

Romero–Gomezcaña, N.R., Sánchez–García, P., Rodríguez–Alcázar, J. and Saucedo–Veloz, C. (2006). Aplicación foliar de calcio y su relación con la calidad en frutos de mango cv. Haden. Calcium foliar sprinkling and its relation with fruit quality of mangoes cv. *Haden. Agricultura Técnica en México*, 32(1), 5-15. https://www.scielo.org.mx/pdf/agritm/v32n1/v32n1a1.pdf

Sayyari, M., Castillo, S., Valero, D., Díaz-Mula, H. M. & Serrano, M. (2011). Acetyl salicylic acid alleviates chilling injury and maintains nutritive and bioactive compounds and antioxidant activity during postharvest storage of pomegranates. *Postharvest Biology and Technology*, 60(2), 136–142. https://doi.org/10.1016/j.postharvbio.2010.12.012

Shivashankara, K.S., Isobe, S., Al-Haq, M.I., Takenaka, M., & Shiina, T. (2004). Fruit antioxidant activity, ascorbic acid, total phenol, quercetin, and carotene of Irwin mango fruits stored at low temperature after high electric field pretreatment. *Journal of Agricultural and Food Chemistry*, 52(5), 1281–1286. https://doi.org/10.1021/jf030243l

Siddiq, M., Sogi, D. S., & Dolan, K. D. (2013). Antioxidant properties, total phenolics, and quality of fresh-cut “Tommy Atkins” mangoes as affected by different pre-treatments. *Food Science and Technology*, 53(1), 156–162. http://doi.org/10.1016/j.lwt.2013.01.017

Singh S. P. & Singh Z. (2012). Role of membrane lipid peroxidation, enzimatic and non-enzymatic antioxidative systems in the development of chilling injury in japanese plums. *Journal of American Society for Horticultural Science*, 136(6), 473-481. https://doi.org/10.21273/JASHS.137.6.473

Sivankalyani, V., Feygenberg, O., Maorer, D., Zaaroor, M., Fallik, E., & Alkan, N. (2015). Combined treatments reduce chilling injury and maintain fruit quality in avocado fruit during cold quarantine. *Plos One*, 10(10), 1–16. https://doi.org/10.1371/journal.pone.0140522

Tomás-Barberán, F. A. & Espín, J. C. (2001). Phenolic compounds and related enzymes as

Revista Bio ciencias
ISSN 2007-3380

determinants of quality in fruits and vegetables. *Journal of the Science of Food Agriculture*, 81(9), 853-876. https://doi.org/10.1002/jsfa.885

Zaharah, S. S., & Singh, Z. (2011). Postharvest nitric oxide fumigation alleviates chilling injury, delays fruit ripening and maintains quality in cold-stored 'Kensington Pride' mango. *Postharvest Biology and Technology*, 60(3), 202-210. https://doi.org/10.1016/j.postharvbio.2011.01.011

Zhao, Z., Jiang, W., Cao, J., Zhao, Y., & Gu, Y. (2006). Effect of cold-shock treatment on chilling injury in mango (*Mangifera indica* L. cv. "Wacheng") fruit. *Journal of the Science of Food and Agriculture*, 86(14), 2458–2462. https://doi.org/10.1002/jsfa.2640