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Effect of UV-C Postharvest Disinfection on the Quality of Fresh-Cut 'Tommy Atkins' Mango

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Mango cv. 'Tommy Atkins' is a highly appreciated fruit for its organoleptic characteristics and its resistance to minimal processing. However, some operations as peeling and cutting can generate microbial contamination and loss of bioactive compounds. Ultraviolet short wave (UV-C) is an alternative technology for fresh-cut products that leads to microbial inactivation and the increase of beneficial compounds. The effect of a UV-C dose of 6 kJ/m^2 was evaluated on quality attributes of fresh-cut 'Tommy Atkins' mango during days 0, 3, 6, 9, and 12 of storage (5°C, relative humidity: 85-90%), and compared with a positive control (conventional method by immersion in 10 mg/L sodium hypochlorite solution) and a negative control (without treatment). Physicochemical analysis (titratable acidity, pH, total soluble solid content, and firmness), superficial color evaluation, determinations of microbial counts, contents of total carotenoids, phenolics and flavonoids, and antioxidant capacity assays were performed. The results showed that UV-C treatment allowed to preserve microbial safety and superficial color of stored fresh-cut mango, and to increase the content of total carotenoids, which was 19.34 and 26.50 mg β-carotene/100 g fresh weight (FW) for control and UV-C treated sample at day 12 of storage, respectively. The DPPH$^•$ scavenging activity of the UV-C treated mango was also higher (0.60 mM TE/g FW) compared to control (0.27 mM TE/g FW) at the end of storage. However, UV-C treatment caused loss of firmness. Some native microorganisms of mango adapted to the stress caused by the treatments and the storage.

Key words: minimal processing, surface color, native microorganisms, predictive microbiology, ultraviolet short wave irradiation

INTRODUCTION

Mango (*Mangifera indica* L.) is a tropical fruit with high production and consumption in the world due to its attractive sensorial attributes and nutritional value [Maldonado-Celis *et al.*, 2019]. It is a source of dietary fiber, vitamins, carbohydrates, fatty acids, and amino acids as well as presents a considerable biological activity by the high content of phenolic compounds, terpenoids, sterols, and carotenoids [Kumar *et al.*, 2021]. The main producing regions of mango in the world are Asia (60% of world production), Latin America (25%), and Africa (14%) [Santo *et al.*,

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2018]. There are more than 1,000 mango cultivars in the world, but the main cultivars harvested in Latin America for commercialization are 'Kent', 'Keitt', 'Ataulfo', 'Tommy Atkins', and 'Haden' [Kansci *et al.*, 2008]. 'Tommy Atkins' cultivar is one of the most appreciated as it is characterized by its large size and color, its resistance to handling, and its potential for minimal processing [Djioua *et al.*, 2010].

Fresh-cut fruits have similar properties to fresh fruits, and their consumption is implied to confer multiple benefits for human health, including the prevention of different chronic, degenerative, and cardiovascular diseases [Artés-Hernández *et al.*, 2017]. However, these products are highly susceptible to microbial growth because their protective tissue is removed and cell contents are released during cutting operation, thereby they serve as a substrate for microorganisms that cause decay and development of human pathogens [Artés-Hernández *et al.*, 2021]. Accordingly, it is important to comply with strict hygiene and manufacturing practices and to recognize critical control points during processing [De Corato, 2020]. For storage, it is necessary to ensure low temperatures and a packaging to control the fresh-cut fruit metabolic activity and accelerated microbial growth. Modified or controlled atmosphere are usually applied using polymeric films and containers, which allows to prevent the flow of respiratory gases, reduce O_2 concentration, and rise CO_2 concentration in the package headspace [Giannakourou & Tsironi, 2021].

With the increase in the consumption of fresh-cut fruits, a concern has arisen regarding the effectiveness of disinfection methods in reducing microorganisms that cause foodborne diseases [Hinojosa *et al.*, 2013]. Different chemical and physical methods have been studied for the disinfection of fresh-cut fruits. The ultraviolet short wave (UV-C) irradiation is an emerging physical technology that inactivates microorganisms and simultaneously induces defense mechanisms in plant. This leads to the production of phytoalexins after treatment and allows to improve the conservation and attributes of minimally-processed fruits [Zambrano-Zaragoza *et al.*, 2021]. These effects depend on the dose of UV-C, although the beneficial effects are attributed to short doses (low dose of radiation or short exposure times).

Different studies have indicated that UV-C treatment preserves quality attributes of fresh-cut mango during storage. González-Aguilar *et al.* [2007] found that irradiation of fresh-cut 'Tommy Atkins' mango for 1 to 10 min induced the accumulation of phenolics and increased antioxidant capacity during storage for 15 days at 5°C. UV-C treatment of 'Kent' mango slices for 30 s had a positive effect on the physicochemical characteristics and produced lower counts of mesophiles, psychrophiles and molds and yeasts, compared to control samples [Márquez-Villacorta *et al.*, 2011]. Irradiation of fresh-cut 'Kent' mango with 14 kJ/m^2 resulted in lower counts of mesophiles and molds and yeasts, and an increment of contents of total phenolics and flavonoids during 15 days of storage at 5°C [Márquez-Villacorta & Pretell-Vásquez, 2013]. However, the treatment doses should be determined empirically for the specific research material. In our preliminary studies, an optimal UV-C dose of 6 kJ/m^2 was estimated for the inactivation of less than 2 log cfu/g of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* on fresh-cut 'Tommy Atkins' mango. The aim of this research was to evaluate the effects of this UV-C dose on the safety and quality attributes of fresh-cut 'Tommy Atkins' mango during refrigerated storage.

MATERIALS AND METHODS

Plant material

Mango fruits (*Mangifera indica* L. cv. 'Tommy Atkins') in stage 3 of maturity were purchased from a Hermosillo (México) local market. Stage of maturity was determined according to the total soluble solid content for this cultivar [Lopes *et al.*, 2021]. Ten kg of fruit with uniform size and maturity were stored at 5°C for 24 h. Subsequently, mangoes were washed, cleaned for 5 min with a 200 mg/L sodium hypochlorite solution, and left to dry in the air.

Fresh-cut mango processing

Peel and seed were removed from fruits. Pulp was cut into 10×60×20 mm spears and divided to three lots. The first lot (C, control) consisted of fresh-cut mango without treatment. The second lot (H) was immersed in a 10 mg/L sodium hypochlorite (NaClO) solution for 1 min and drained for 2 min [Djioua *et al.*, 2010]. A UV-C dose of 6 kJ/m^2 established by Rodríguez-Mijangos *et al.* [2014] was applied to the third lot (UV-C treatment) in a portable disinfection chamber. Groups of three spears were arranged in the center of the equipment at 75 mm from the radiation source for 26 min and 48 s. Approximately 90 g of fresh-cut fruit from each lot were distributed in polyethylene terephthalate (PET) containers (12 cm long, 10 cm wide, and 6 cm high). Fifteen containers per each lot were used for quality analyses. Fresh-cut mango was stored under refrigeration at 5°C with a relative humidity (RH) of 85-90% for 12 days.

Analyses of pH, titratable acidity, total soluble solid content, and firmness

Titratable acidity (TA), pH, total soluble solid (TSS) content, and firmness analyses were performed. pH was measured with a potentiometer (STARTER 2100, OHAUS, Nänikon, Switzerland). TA was assessed by titration with 0.1 N NaOH (Thermo Fisher Scientific, Waltham, MA, USA) considering citric acid as a predominant organic acid in mango. TSS content was determined according to International AOAC 932.12 method using a manual refractometer [AOAC, 2012]. Firmness was measured with a texture analyzer (EZ-S, Shimadzu, Kyoto, Japan) through a Warner-Bratzler shear force test following the methodology proposed by Lázaro & De Lorenzo [2015] with some modifications. The mango samples were cut crosswise with a 2 mm thick Warner-Bratzler steel blade at a test speed of 100 mm/s. Firmness was considered as the maximum force applied to break a piece of mango.

Measurements of color parameters

Mango surface color parameters were evaluated with a MiniScan XE plus portable colorimeter (HunterLab, Reston, VA, USA) in

CIELab tristimulus coordinates: L^* (lightness: white(+)/black(–)), a^* (red(+)/green(–)), and b^* (yellow(+)/blue(–)). Equipment was calibrated with a reflector plate (X=80.1, Y=85, Z=90.6) considering a standard illuminant D65 and a 10° observer. Chroma (C^*), hue angle ($h°$), and total color difference (ΔE) were calculated with the following formulas [Pathare *et al.*, 2013]:

$$C^* = (a^{*2} + b^{*2})^{\frac{1}{2}} \quad (1)$$

$$h° = tan^{-1}(b^* / a^*) \quad (2)$$

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{\frac{1}{2}} \quad (3)$$

Microbial count analysis

According to Hinojosa *et al.* [2013], three randomized samples of 15 g for each treatment were individually homogenized in 135 mL of 0.1% peptone water (Difco, Fisher Scientific, Hampton, NH, USA) in a sterile stomacher bag (Nasco, Whirl-Pak, Madison, WI, USA) for 2 min. Serial dilutions were prepared with 0.1% peptone water. For each dilution, aliquots of 1 and 5 mL were plated in triplicate on different mediums (a total of six Petri dishes per sample). Plates with plate count agar (Difco) and violet red bile agar (BD, Franklin Lakes, NJ, USA) were incubated for approximately 48 h (Analog Incubator 132000, Boekel Scientific, Feasterville, PA, USA) for aerobic mesophilic and total coliform counts, respectively. Plates with plate count agar and potato dextrose agar (Difco) were incubated for 7 days at 5°C and 22°C to count psychrotrophic bacteria and yeast and molds, respectively. The results were expressed as log colony forming units (cfu) per g of fresh-cut mango. The microbial quality of fresh-cut mango was evaluated following Colombian microbial legislation for minimally processed fruits [Ministry of Health and Social Protection of Colombia, 2013].

Inactivation kinetic modelling

A model of inactivation of microorganisms was developed using the linear logarithmic equation and Weibull model as a function of storage days [van Boekel, 2002] using formulas (4) and (5), respectively.

$$\log(N / N_0) = -kt / 2.303 \quad (4)$$

$$\log(N / N_0) = (-1 / 2.303)(t / \alpha)^{\beta} \quad (5)$$

where: N_0 and N are the numbers of initial and surviving microorganisms, respectively, and t corresponds to time of storage (day), k is the inactivation rate constant (1/day), α is the characteristic time (day), and β is the shape factor.

The inactivation parameters were estimated with the averages of the logarithmic reductions of the microbial populations during the storage time. Linear least squares method and non-linear least squares method with the resolution of the Gauss-Newton algorithm (maximum number of iterations of 200 and a convergence tolerance of 0.00001) were used to fit the inactivation data of microorganisms to the logarithmic linear equation and to the Weibull model, respectively. The fit of the models was evaluated by calculating the coefficient of determination (R^2), the adjusted coefficient of determination (adjusted R^2), and the root mean square error (RMSE).

Extract preparation

Two grams of mango samples were added to 3 mL of absolute ethanol. The solutions were mixed (MX-S vortex mixer, Science Med, Helsinki, Finland), sonicated for 30 min (Branson 1800 Digital Bath, Emerson, St. Louis, MO, USA), and placed in refrigeration and darkness for night. Subsequently, these solutions were centrifuged at 257×*g* for 20 min (Compact II centrifuge, BD). The supernatant was used for the quantification of bioactive compounds.

Total carotenoid content determination

Following the method proposed by González-Vega *et al.* [2021], 300 µL of each extract were added in triplicate to wells of the microplate, and absorbance was measured at 450 nm in a 96-well microplate reader (Multiskan GO, Thermo Fisher Scientific). A calibration curve was plotted with concentrations between 0.002 and 0.1 mg/mL of β-carotene standard (Sigma-Aldrich, St. Louis, MO, USA) (R^2=0.991). Results were reported as mg β-carotene equivalents per 100 g of fresh weight (FW) of mango.

Determination of total phenolic and flavonoid contents

The total contents of phenolics and flavonoids were determined following Pérez-Perez *et al.* [2021] methodology. For total phenolic content, 10 µL of each extract and 25 µL of 0.2 M Folin-Ciocâlteu reagent solution (Sigma-Aldrich) were initially added to each well of the microplate. The mixture was left under refrigeration for 5 min and subsequently, 25 µL of 20% Na_2CO_3 and 140 µL of distilled water were added. The microplate was put to rest for 30 min, and the absorbance was measured at 760 nm on the microplate reader. A calibration curve was plotted with concentrations between 0.1 and 1 mg/mL of gallic acid standard (Sigma-Aldrich) (R^2=0.992). The results were expressed as mg gallic acid equivalents (GAE) per 100 g of FW of mango.

For total flavonoid content determination, 80 µL of each extract and 80 µL of an ethanolic solution of aluminum trichloride (20 g/L) were added to wells of the microplate. The microplate was shaken for 30 s and left in the dark for 1 h at 25°C. Subsequently, it was shaken once more for 30 s, and the absorbance was measured at 415 nm in the microplate reader. The calibration curve was plotted with the quercetin standard (Sigma-Aldrich), considering concentrations from 0 to 0.08 mg/mL (R^2=0.999). The results were reported as mg quercetin equivalents (QE) per 100 g of FW of mango.

Antioxidant capacity assays

Assays were performed following the methodology reported by Pérez-Perez *et al.* [2021] with some modifications. To determine the antioxidant capacity by scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 1 mg of the DPPH radical (Sigma-Aldrich)

was weighed and dissolved in 1 mL of absolute ethanol. From this initial solution, 0.75 mL was added to 29.25 mL of ethanol. The absorbance of the resulting solution was adjusted to 0.7 at a wavelength of 515 nm. Next, 200 µL of the resulting solution and 20 µL of the extract were added to wells of the microplate. The absorbance was measured at 515 nm in the plate reader after 30 min of leaving the microplate to rest. A calibration curve was made with Trolox standard (Sigma-Aldrich) considering concentrations between 0 and 0.2 mg/mL (R^2=0.992). The results were expressed as mM Trolox equivalents (TE) per g of FW of mango.

For determination of the antioxidant capacity by 2,2′-azino--bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, 19.3 mg of ABTS were dissolved in 5 mL of distilled water. Separately, 0.0378 g of potassium persulfate and 1 mL of distilled water were mixed. From the second solution, 88 µL were added to the first solution, and it was left to rest in the dark for 16 h under refrigeration. One mL of this last prepared solution containing generated ABTS radical cations was added to 88 mL of ethanol, and the absorbance was adjusted to 0.7 at a wavelength of 734 nm. For the measurement of the samples, 270 µL of the ABTS radical cation solution and 20 µL of each extract were taken. The absorbance was measured at 734 nm after 30 min of rest. The calibration curve was plotted with Trolox standard considering concentrations from 0 to 0.01 mg/mL (R^2=0.994). The results were expressed as mM TE/g FW.

For ferric reducing antioxidant power (FRAP) determination, the stock solutions were prepared: a 300 mM sodium acetate buffer at pH 3.6, a 20 mM $FeCl_3 \times 6H_2O$ solution, and a solution of 10 mM 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ; Sigma-Aldrich) in 40 mM HCl. The working solution was obtained by adding the stock solutions in a 10:1:1 (*v/v/v*) ratio (buffer, $FeCl_3 \times 6H_2O$, and TPTZ-HCl, respectively). Then, 280 µL of the working solution and 20 µL of each extract were added to each well of the microplate. Absorbance was measured at 638 nm after 30 min of rest. The calibration curve was plotted with Trolox standard considering concentrations between 0 and 0.4 mg/mL (R^2=0.999). Results were reported as mM TE/g FW.

Statistical analysis

A completely randomized design with two factors and a 3×5 factorial arrangement was used. The first factor (A) was the treatment of disinfection of fresh-cut mango with three levels (without disinfection treatment or control (C), immersion in 10 mg/L sodium hypochlorite solution (H), and application of a UV-C dose of 6 kJ/m^2 (UV)). The second factor (B) corresponded to the sampling time during storage of fresh-cut mango in refrigeration, comprising five levels (0, 3, 6, 9, and 12 days). Fresh-cut mango from the control treatment was considered as a negative control, and from the treatment with NaClO as a positive control. Results were presented as the mean of three replicates. A two-way ANOVA and a Tukey's test were performed to compare mean values between treatments and evaluate the effect of factors on pH, TA, TSS content, firmness, color parameters, microbial counts, contents of total carotenoids, total phenolics and flavonoids, and antioxidant capacity of fresh-cut mango (p<0.05).

RESULTS AND DISCUSSION

Titratable acidity, pH, total soluble solid content, and firmness

The results of determinations of titratable acidity, pH, total soluble solid content, and firmness of mango treated with UV-C, immersed in sodium hypochlorite solution and without treatment during storage are shown in Figure 1. According to the two-way ANOVA, the disinfection treatment, the storage time, and the interaction between these factors had a significant effect on the physicochemical properties of fresh-cut mango (p<0.05). Values of pH indicated that fresh-cut mango from C (3.88–4.18) and H (3.75–3.95) treatments was entering the senescence stage. For mango irradiated with UV-C, pH started to decrease significantly since day 9 of storage. According to Zambrano-Zaragoza *et al.* [2021], UV-C treatment allows delaying senescence stage. The content of TSS of fresh-cut mango from UV treatment tended to increase until day 9 of storage. The increase in TSS content is attributed to the hydrolysis of polysaccharides and the accumulation of released soluble sugars as a result of fruit ripening during storage [George *et al.*, 2015; Khubone & Mditshwa, 2018]. On the other hand, TSS content of C sample remained almost constant and its content of H sample decreased slightly only at day 6 (14.13 °Brix) of storage (Figure 1). Treatments could affect the rate of transpiration, generating slight changes in the content of water in the fruit [Zambrano-Zaragoza *et al.*, 2021]. The titratable acidity of fresh-cut mango from C treatment exhibited an increase up to day 9 of storage (0.48–0.85 g/100 g) and then decreased slightly (to 0.76 g/100 g) (Figure 1). The reduction in acidity levels can be initially linked to the ripening of the fruit when organic acids are degraded [Barreto *et al.*, 2021]. For the samples from H and UV treatments, TA increased until day 3 of storage and then remained almost constant (Figure 1). The disinfection treatments and the modified atmosphere could slow down the respiration rate, and the utilization rate of the respiratory substrate was minimal through the storage period [Razali *et al.*, 2021].

The firmness of fresh-cut mango tended to decrease during refrigerated storage (Figure 1). According to Barreto *et al.* [2021], the reduction of firmness of minimally processed fruits is generated by the cutting operation and the high degree of injury to cellular tissues. The demethylation and depolymerization of pectin, generation of free radicals, enzymatic degradation of hemicellulose, solubilization of polyuronides, the release of galactose from pectic polymers, cell wall swelling, decreased water content and osmotic potential may occur in tissues and thus lower the fruit firmness [Barreto *et al.*, 2021; Márquez-Villacorta & Pretell-Vásquez, 2013]. In our study, it was found that mango irradiated with UV-C exhibited the lowest firmness for most of the storage time (2.10–5.63 N) compared to control (3.53–4.46 N) and H-treated (2.03–5.94 N) samples (Figure 1). Fruit softening is modulated by cell wall hydrolases,

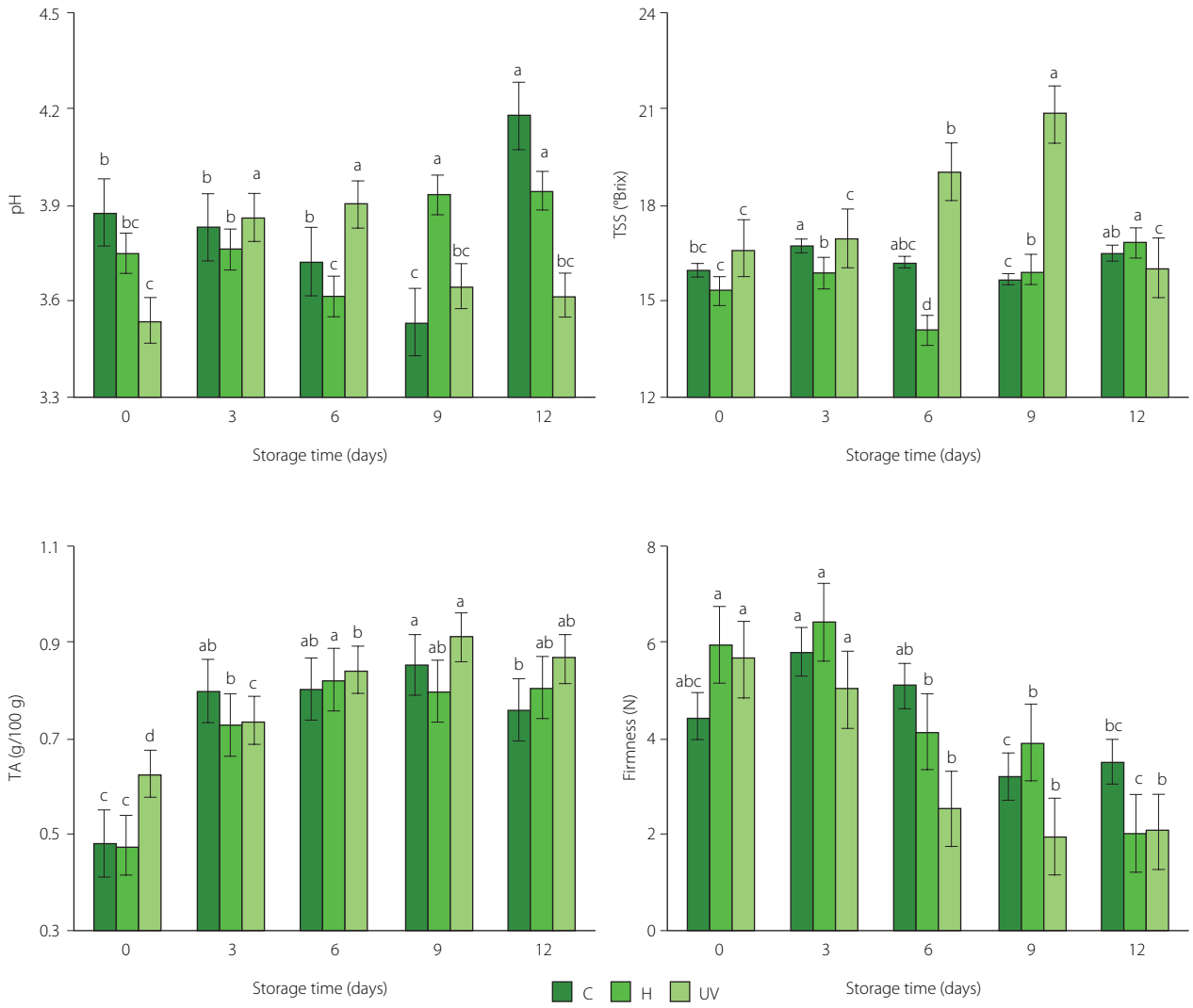

FIGURE 1. Values of pH, total soluble solid (TSS) content, titratable acidity (TA), and firmness of fresh-cut mango without treatment (control, C), immersed in sodium hypochlorite solution (H), and treated with ultraviolet short wave (UV-C, UV) during storage at 5°C for 12 days. Vertical lines above and below the points correspond to standard error. Different lowercase letters (a-d) above bars (separately for control and each treatment) indicate significant differences ($p<0.05$).

such as polygalacturonase, cellulase, β-galactose, and expansin, and UV-C treatment can alter the expression of genes involved in modifying cell wall structure [Kan *et al.*, 2021].

Color parameters

It was found that the disinfection treatment had no significant effect on *L** coordinate of fresh-cut mango ($p\geq0.05$). However, the storage time and the interaction of the factors had a significant effect on the values of this parameter ($p<0.05$). In Table 1, it is shown that *L** color coordinate of mango samples from C and UV treatments tended to increase slightly during storage, and to decrease for the samples from H treatment. Luminosity is considered an indicator of browning. The *L** value of the H-treated fresh-cut mango decreased during the storage possibly due to sample browning as a result of oxidation processes induced by NaClO. The disinfectants served as oxidizing agents [Hinojosa *et al.*, 2013]. In the case of UV-C treatment, inhibition of polyphenol oxidase (PPO) activity by radiation could occur [Barreto *et al.*, 2021] and result in the reduced browning of the fruits.

The disinfection treatment, the storage time, and the interaction between these factors had a significant effect on all other color parameters of fresh-cut mango ($p<0.05$). Low values of *a** coordinate represented tones between green and red. The values of *a** increased for the control sample, decreased for the samples from H treatment, and remained slightly constant for the samples from UV treatment during storage (Table 1). The increase in *a** coordinate value can be interpreted as the increase in orange or red pigments due to fruit ripening, and on the other hand, as enzymatic and non-enzymatic browning [Márquez-Villacorta & Pretell-Vásquez, 2013; Nguyen *et al.*, 2022]. Therefore, the radiation treatment allowed delaying some adverse processes linked to accelerated ripening and browning of the fruit, which possibly occurred in mango from C and H treatments, respectively. Positive values of *b** coordinate indicated yellow tones. This coordinate tended to remain constant for the samples from H and UV treatments during storage (Table 1). During mango ripening, green chloroplasts become chromoplasts with red and yellow tones. These tones in mango are mainly derived