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Original Article

Influence of chitosan coating with mint essential oil on the microbiological incidence, bioactive compounds, and antioxidant capacity of fresh-cut mango

Influencia del recubrimiento de quitosano con aceite esencial de menta sobre la seguridad microbiológica, los compuestos bioactivos y la capacidad antioxidante del mango fresco cortado

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ABSTRACT

Fresh-cut mango is in high demand in developed countries; however, it deteriorates quickly and represents a suitable medium for microbial growth during storage. Chitosan coatings and other natural materials, such as essential oils, have been used to lessen these issues. The aim of this study was to evaluate the effect of chitosan (CH), mint essential oil (MEO) and their combination (CH+MEO) on the microbiological incidence, content of bioactive compounds, and antioxidant capacity of fresh-cut mango stored during 15 d at 5 °C. Microbiological analyses (mesophilics, psychrophilics, molds, and yeasts) were evaluated after 0, 7 and 15 d of storage. Physical (color and firmness) and chemical (total soluble solids, titratable acidity, ascorbic acid, phenols, β-carotene, and antioxidant capacity) analyses were performed every 3 d. The applied treatments successfully reduced microbial growth with the same intensity, furthermore, none of them caused significantly negative effects on color, firmness, total soluble solids, titratable acidity, and ascorbic acid. However, the highest total phenolics and β-carotene contents were obtained by CH+MEO, while fruit treated with CH and CH+MEO had the highest antioxidant capacity. The application of CH+MEO could be used to reduce its microbial load while increasing bioactive compounds and antioxidant capacity of fresh-cut mango.

Keywords: Minimally processed mango; antimicrobial treatments; DPPH; physicochemical quality; *Mangifera indica*

RESUMEN

El mango fresco-cortado tiene gran demanda en países desarrollados; sin embargo, éste se deteriora rápidamente y presenta un medio adecuado para el crecimiento microbiano durante el almacenamiento. Los recubrimientos de quitosano y otros materiales naturales, como los aceites esenciales, han sido utilizados para disminuir estos daños. El objetivo de este estudio fue evaluar el efecto del quitosano (CH), aceite esencial de menta (MEO) y su combinación (CH+MEO) sobre la incidencia microbiológica, el contenido de compuestos bioactivos y la capacidad antioxidante del mango fresco-cortado almacenado durante 15 d a 5 °C. Los análisis microbiológicos (mesófilos, psicrófilos, hongos y levaduras) se evaluaron después de 0, 7 y 15 d de almacenamiento. Los análisis físicos (color y firmeza) y químicos (sólidos solubles totales, acidez titulable, ácido ascórbico, fenoles, β-caroteno y capacidad antioxidante) se realizaron cada 3 d. Los tratamientos aplicados redujeron con éxito el crecimiento microbiano con la misma intensidad, además ninguno causó efectos negativos significativos en color, firmeza, sólidos solubles totales, acidez titulable y ácido ascórbico. No obstante, los contenidos más altos de fenoles totales y β-caroteno se obtuvieron con CH+MEO, mientras que los frutos tratados con CH y CH+MEO tuvieron la mayor capacidad antioxidante. La aplicación de CH+MEO podría usarse para reducir su carga microbiana al mismo tiempo que aumenta los compuestos bioactivos y la capacidad antioxidante del mango fresco-cortado.

Palabras clave: Mango mínimamente procesado, tratamientos antimicrobianos, DPPH, calidad fisicoquímica, *Mangifera indica*

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important fruits worldwide due to its organoleptic characteristics and its high content of bioactive compounds with antioxidant capacity (de Oliveira *et al*., 2017). The consumption of fresh-cut mango is increasing due to changes in eating habits related with health concerns and the lack of time to prepare food (Rico-Rodríguez *et al*., 2015). However, fresh-cut fruits are highly exposed to the attack of microorganisms because the cutting process provokes the release of cellular contents, which favor the microbial growth and reactions affecting the

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physicochemical properties of the fruit (Chen *et al*., 2022). Hence, efficient conservation methods are needed to lessen these deterioration processes and preserve the quality attributes (Alikhani, 2014; Ayón-Reyna *et al*., 2015).

In recent years, the use of essential oils on fruits has received attention as a possible natural alternative to improve fruit microbiological quality and shelf-life (Sarengaowa *et al*., 2022). The essential oil of *Mentha piperita*, also known as mint essential oil (MEO), is considered as non-toxic, non-irritant and contains a wide variety of chemical components with antimicrobial and antioxidant properties (Tafrihi *et al*., 2021). Also, the U.S. Food and Drug Administration has recognized this essential oil as a GRAS (Generally Regarded as Safe) food additive (FDA 21CFR182.20) (FDA, 2023). However, the active components of essential oils easily evaporate and degrade by light, heat, and oxidation reactions, affecting their stability and functionality, and for these reasons, in many cases it is not recommended to use essential oils in their free form (Sotelo-Boyás *et al*., 2015). In order to improve stability and maintain the functionality of the bioactive compounds, essential oils are incorporated into edible/biodegradable films, like chitosan-based films (CH), to encapsulate them (Grande-Tovar *et al*., 2018). Chitosan is a biodegradable and non-toxic polysaccharide that is applied on fruits as an edible coating, acting as a semi-permeable barrier that protects the fruit surface, helping to reduce moisture loss and preventing the contamination of fruit (Grande-Tovar *et al*., 2018; Martínez *et al*., 2018). Also, chitosan has been reported to have a very broad spectrum of antimicrobial activity, controlling the growth of several phytopathogenic fungi and bacteria (Sotelo-Boyás *et al*., 2015; Sarengaowa *et al*., 2022).

An effective treatment to extend fresh-cut fruit shelf-life could be obtained if the antifungal and barrier properties of chitosan are combined with the antimicrobial properties of MEO (Martínez *et al*., 2018). Some examples of essential oils that have been combined with chitosan and applied to fresh-cut fruit and vegetables (e.g., mango, lettuce, cantaloupe, etc.) include marjoram, orange, and ginger essential oils (Rico-Rodríguez *et al*., 2015; Xylia *et al*., 2021; Chen *et al*., 2022). In this sense, Chen *et al*. (2022) registered a growth reduction in molds and yeasts by the application of chitosan and ginger essential oil in fresh-cut cantaloupe. Also, in a study reported by Jovanovic *et al*. (2016) the effect of chitosan alone or combined with mint or thyme oil was evaluated on the growth of *Listeria monocytogenes*, observing a strong antimicrobial activity by chitosan which was increased by the addition of essential oils.

Despite the fact that MEO in combination with chitosan has already been applied to whole fruits and vegetables such as table grapes (Dantas-Guerra *et al*., 2016), cabbage (Jovanovic *et al*., 2016), mangoes (de Oliveira *et al*., 2017), and papayas (Dos Passos Braga *et al*., 2019), currently there are no studies on the impact of the application of this combination of compounds, CH+MEO, on the microbiological incidence, bioactive compounds, and antioxidant properties of fresh-cut mango. The objective of this study was to evaluate the effect of CH, MEO, and CH+MEO on the quality of fresh-cut mango during refrigerated storage.

MATERIALS AND METHODS

Materials

Mango fruit (*Mangifera indica* L. cv. Kent) at maturity index 5 (firmness of approximately 16 N and 15° Brix) was obtained from a local producer in Culiacan, Sinaloa, Mexico. The fruits included in the experiment did not show physical damage and had a size between 500 and 700 g.

Mint essential oil (*Mentha piperita* L.) was acquired from Aceites y Esencias, S.A., Mexico City, Mexico. Food-grade chitosan (86 % deacetylation degree) was acquired in Agrinos, S.A., Etchojoa, Sonora, Mexico.

Edible coatings preparation

Chitosan (CH) was dissolved (1 %, w/v) in deionized water at 40 °C, adding glacial acetic acid (1 %, v/v) under shaking conditions for 12 h (Aloui *et al*., 2014). The mint essential oil (MEO) was dispersed in deionized water (0.02 %, v/v) at 35 °C min using a homogenizer (T18 Basic Ultra-Turrax, IKA, Oxford, England) at 13,500 rpm for 5. The MEO dispersion was added to the CH solution (1 %, w/v) until a final concentration of 0.02 % (v/v), homogenizing at 13,500 rpm for 4 min (Ali *et al*., 2015). All solutions were adjusted to pH 5.6 by adding NaOH (1 M) and were added with 1 % Tween 80® to stabilize them for at least 24 h.

Fruit processing

Mango fruit were washed and sanitized by immersion in a solution of sodium hypochlorite (300 µL/L) for 5 min. Subsequently, fruits were peeled and transversely cut into 1 cm thick slices inside a room at 5 °C. The slices were divided into 4 lots: one lot was not treated (control), another lot was treated by immersion in the CH solution (1 %, w/v), the third lot was treated by immersion in the MEO solution (0.02 %, v/v), and the fourth lot was treated by immersion in the CH+MEO emulsion. All immersions were performed at 5 °C for 3 min.

Seven to nine slices of treated mango (200 g) were placed into 0.25 L hermetic polystyrene trays with flat lid (Nutrigo S.A. de C.V., Mexico City, Mexico) and stored for 15 days at 5 °C. Three trays per treatment were removed for microbiological analysis on days 0, 7, and 15; while for physical and chemical analysis 3 trays were evaluated every 3 days. For physical quality analyses, three slices per tray were evaluated, while for chemical analyses, bioactive compounds and antioxidant capacity, the rest of the slices were chopped into small cubes and a mixture was made from which 3 samples were taken per tray for each parameter. Three replicates per treatment were performed.

Microbiological analysis

Microbiological analysis was carried out according to Ayón-Reyna *et al*. (2015). A sample of 50 g of untreated or treated mango slices were homogenized with 450 mL of 1 % peptone water for 1 min (1:10 dilution) and then 1 mL of the homoge-

nized sample was mixed with 9 mL of Brain Heart Infusion broth (Bioxon BD, Sparks, Md, USA) (1:100 dilution). Mesophilic and psychrophilic counts were evaluated by placing 100 µL of each dilution into plates count agar. Plates were incubated for 24-48 h at 37 °C for the isolation of mesophilic microorganisms, and for 10-15 days at 5 °C for the isolation of psychrophilic microorganisms. Molds and yeasts count was carried out by placing 100 µL of each dilution into plates containing Sabouraud agar and then ncubated for 3-5 days at 25 °C. Six measurements were performed per treatment and the results were reported as colony forming units per gram (CFU/g).

Physical quality analysis

The color of the mango slices (L* and b*) was evaluated at the center of each slice using a CR-200 colorimeter (Minolta Co. Ltd., Osaka, Japan). One measurement was taken for each slice and three measurements per tray were performed, evaluating a total of 9 slices per replicate (Ayón-Reyna *et al*., 2015).

Firmness was evaluated at the center of each slice using a Chatillon penetrometer (DFE 100; AMETEK Inc., Largo, Fl., USA) fitted with an 11 mm diameter cylindrical probe with a speed of 50 mm/min and a penetration deep of 5 mm (Ayón-Reyna *et al*., 2015). Results were reported as Newton (N). Nine measurements were made per treatment.

Chemical quality analysis

Total soluble solids (TSS) were evaluated according to Ayón-Reyna *et al*. (2017) using a refractometer (Atago, Fisher Scientific, GA, USA) and results were expressed as °Brix. For titratable acidity (TA), mango slices (20 g) were homogenized with distilled water (100 mL) at pH 7 and filtered using organza cloth. The titration method was used and the results were expressed as percentage of citric acid (Ayón-Reyna *et al*., 2017).

Bioactive compounds analysis

Ascorbic acid was analyzed following the methodology described by López-Velázquez *et al*. (2020). A 20 g sample of mango slices was homogenized with deionized water and filtered using Whatman filter No. 1, followed by a filtration with 0.45 µm pore size disposable filters and by Sep-Pak C-18 cartridges. Ten µL of filtered solution were analyzed in a 1100 HPLC system (Agilent, Waldbronn, Germany) equipped with a Sphereclone ODS2 column (250 mm x 4.6 mm x 5 µm; Phenomenex, U.S.A.) operated at 16 °C. Monobasic potassium phosphate (25 mM) was used as mobile phase at a 0.7 mL/min flow rate. Ascorbic acid was monitored at 254 nm. A calibration curve was used to determine the ascorbic acid content and results were expressed as mg/g of fresh weight (FW).

Total phenols were determined according to Ayón-Reyna *et al*. (2018) with some modifications. Methanol extracts were obtained by homogenizing 2.5 g of mango slices, 12.5 mL methanol (80 %), and sodium bisulfite (0.5 %) using an Ultra-Turrax. Subsequently, the homogenate was sonicated for 30 min and centrifuged at 1500 rpm for 10 min at 4 °C. The supernatant was filtered and the volume adjusted to 4 mL with methanol (80 %). A 20 µL aliquot of methanol extract was mixed with 180 µL of Folin-Ciocalteu reagent and 50 µL of 7 % sodium carbonate. The mixture was allowed to stand for 30 min in darkness at 40 °C, and the absorbance was measured at 765 nm using a microplate reader (Synergy HT, BioTek Instruments, Winooski, VT, USA). A standard calibration curve of gallic acid (0-500 µg/mL) was used and the results were expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of fresh weight (mg GAE/g FW).

β-carotene was determined homogenizing 10 g of mango slices with 20 mL of acetone and filtered using organza cloth followed by a 0.45 µm pore size syringe filter (PVDF membrane HPLC Certified, Pall, Parsippany, MI, USA). An aliquot of 100 µL was analyzed in a 1100 HPLC system (Agilent, Waldbronn, Germany) equipped with a YMC carotenoid column (250 mm x 4.6 mm x 5 µm; YMC Co. Ltd., Kyoto, Japan). A mobile phase of 15 % of methyl *tert*-butyl ether, 81 % of methanol and 4 % of water was used, with a flow rate of 0.7 mL/min. β-carotene was monitored at 447 nm and the results were reported as mg of β-carotene/g FW (Ayón-Reyna *et al*., 2018).

Antioxidant capacity analysis

The antioxidant capacity determined by DPPH (2, 2'-diphenyl-1-picrylhydrazyl) method was performed mixing 0.1 mL of methanol extract (obtained in the same way as for phenolic compounds) with 2.9 mL of the 150 mM DPPH solution in methanol, and incubated in the dark for 30 min at room temperature. A microplate reader (Synergy HT, BioTek Instruments, Winooski, Vermont, USA) was used to measure the absorbance at 525 nm. A calibration curve was prepared with Trolox (25-225 µg/mL) and the results were expressed as µmol trolox equivalent/g FW (Ayón-Reyna *et al*., 2018).

Statistical analysis

A completely randomized experimental design with 3 replicates per treatment (each replicate consisting of 3 trays with 200 g of mango slices) was performed considering treatment and storage time as factors. Data were analyzed through multiple analyses of variance using Statgraphics Plus 5.1 and the means were compared using Fisher's Least Significant Difference (LSD) test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Microbiological analysis

Mesophilic bacterial growth in mango slices was not detected at the beginning of storage at any treatment; however, the mango slices of the control showed their presence at 7 and 15 days of storage, showing at this last storage time a marked increase in bacterial growth with corresponding values of 2.8×10^7 CFU/g. On the other hand, with the application of CH, MEO, and CH+MEO treatments, there were not mesophilic microorganisms after 7 days and notably lower

bacterial content after 15 days of storage compared to the control one (Figure 1A).

A similar behavior was observed in the count of psychrophilic microorganisms. The initial growth of psychrophiles in mango slices was very low in all treatments (1000 CFU/g) (Figure 1B). At 7 days of storage no significant changes were observed, while the control treatment presented a slight increase. It was observed that the control treatment had the highest development of psychrophilic microorganisms at the end of storage (3.2 x 10^7 CFU/g) while CH, MEO, and CH+MEO treatments had very low CFU (1.3 x 10^5, 8.9 x 10^4 and 2. x 10^5 CFU/g, respectively).

The results obtained for yeasts and molds are shown in Figure 1C. The mango slices treated with CH, MEO, and CH+MEO maintained low microbial loads during the first 7 days, followed by a slight but not significant increase at day 15. These treatments did not show significant differences between them during the complete storage time. On the other hand, untreated slices presented a continuous increase in the development of yeasts and molds, reaching a high average value at the end of the storage (5.5 x 10^6 CFU/g).

Previous reports have shown a beneficial effect of chitosan as antimicrobial agent against several postharvest diseases in whole fruit (Liu *et al*., 2007; López-Zazueta *et al*., 2023) as well as in the development of bacterial, yeasts and molds in fresh-cut fruit (Ayón-Reyna *et al*., 2015; Abdelgawad *et al*., 2022; Che *et al*., 2022). For instance, Minh *et al*. (2019) reported a significant reduction in the total bacterial count in fresh-cut pineapples coated with 0.25 % chitosan during 21 days of storage, which coincides with what was found in the present study since chitosan decreased the development of mesophilic and psychrophilic bacteria.

Additionally, several studies proved the inhibitive effect of essential oils on the microbial load of fresh-cut fruit (Alikhani, 2014; Viacava *et al*., 2018). In this sense, mint essential oil (0.01-0.08mL/L) revealed antimicrobial effect on the survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* inoculated into fresh-cut lettuce and purslane, and observed that the concentration of essential oil and the exposure time affects microbial development (Karagözlü *et al*., 2011).

Mint essential oil has been shown as an effective antimicrobial agent because of its menthol and menthone components, which present a good inhibitory effect against gram positive and negative bacteria, yeast, and molds (Picard *et al*., 2013; Abdelgawad *et al*., 2022).

The antimicrobial mechanism of chitosan can be explained in different ways. Some authors have suggested that cationic chitosan interact with the negative charges of the microbial surfaces, resulting in a breakdown of cell membranes, causing leakage of intracellular constituents, hindering vital microbial activities, and thus provoking cell death (Raafat *et al*., 2008). Furthermore, it has also been suggested that chitosan oligomers can penetrate microbial cells and affect RNA transcription and protein synthesis (Jovanovic *et al*., 2016). Additionally, chitosan is the main component of the cell walls of fungi, so when chitosan is applied to the fruit, a defense mechanism is activated because the positive charges of the NH_3^+ groups of chitosan interact with negatively charged pectin, affecting their molecular structure, thus producing an alarm signal that informs plant cells that their cell walls are being degraded by fungi (Stasińska-Jakubas and Hawrylak-Nowak, 2022).

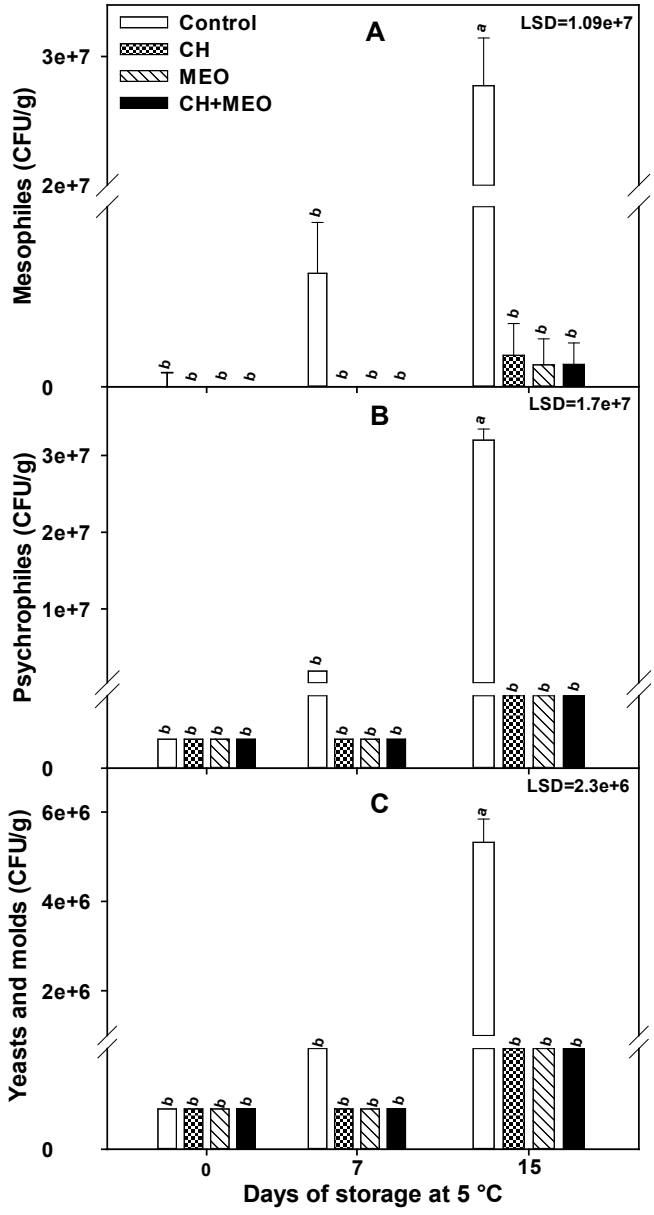

Figure 1. Presence of mesophilic (A), psychrophilic (B), yeasts and molds (C) microorganisms during a 15-day storage of mango slices at 5 °C, coated with chitosan (CH), mint essential oil (MEO), and the combination (CH+MEO). Vertical bars represent standard deviation of the mean. Different letters indicate significant differences among treatments and days. Fisher's Least Significant Difference (LSD) test ($p \leq 0.05$).

Figura 1. Presencia de microorganismos mesófilos (A), psicrófilos (B), levaduras y mohos (C) durante el almacenamiento a 5 °C de mango recién cortado recubierto con quitosano (CH), aceite esencial de menta (MEO) o su combinación (CH+MEO). Las barras verticales en las columnas representan la desviación estándar de las medias de tres réplicas. Las letras diferentes indican diferencias significativas entre tratamientos y días.

Even though in the present study the incorporation of essential oil in chitosan coating did not improve its antimicrobial activity, Abdelgawad *et al*. (2022) observed that it depends on the type of oil employed. Authors observed that 0.5 % chitosan coating alone or combined with 0.5 % peppermint essential oil reduced microbial growth in fresh-cut green beans pods compared to the control (untreated); however, the combination of peppermint oil with chitosan had no significant differences to chitosan after 15 days of storage, but when combined with 0.5 % tea tree oil, the total bacterial count was reduced. The antimicrobial action of essential oils is due to their chemical components, including compounds like menthol and menthone in mint essential oil, and 4-terpinenol in tea tree oil, which have different mechanisms of action, because depending on the compounds present, some oils may be more effective at disrupting the cell membrane, while others may interfere inhibiting ergosterol biosynthesis or causing changes in the cytoplasm. Also, other factors such as the geographical origin of the plant, the growing conditions and the essential oil extraction process also influence its antimicrobial activity (Chouhan *et al*., 2017).

The population of microorganisms of the treated mango slices is within the scale contemplated as natural microbiota of a fresh-cut produce (about 10^5 CFU/g) (Ayón-Reyna *et al*., 2015; NOM-093). Also, Alikhani (2014) reported that the critical limit for total aerobic plate count for vegetables is 10^8 CFU/g.

Physical properties

The lightness (L*) values of mango slices remained constant during the first nine days of storage in all treatments, followed by a decrease during the rest of the storage period, becoming less bright (Table 1). At day 15, slices treated with MEO had the highest L* values, while the ones treated with CH and CH+MEO had the lowest values, which were not statistically different to the control. The retention of lightness in fresh-cut mango treated with the MEO could be due to delayed moisture loss, as reported by Barreto *et al*. (2016) in

Table 1. Fruit quality of fresh-cut mango coated with chitosan (CH), mint essential oil (MEO), and the combination (CH+MEO) stored at 5 °C during 15 days.
Tabla 1. Luminosidad, valor b*, firmeza, sólidos solubles totales (SST) y acidez titulable (AT) en mango fresco cortado recubierto con quitosano (CH), aceite esencial de menta (MEO) o su combinación (CH+MEO), y almacenado a 5 °C.

Treatments	Days of storage at 5 °C					
	0	3	6	9	12	15
			Lightness (L)			
Control	71.59 ± 1.29^{a}	70.77 ± 4.08^{a}	71.98 ± 3.86^{a}	71.45 ± 2.55^{a}	68.81 ± 4.73^{a}	66.19 ± 2.81^{ab}
CH	70.57 ± 2.08^{a}	70.56 ± 1.64^{a}	72.41 ± 2.33^{a}	72.65 ± 5.06^{a}	67.89 ± 2.72^{a}	63.61 ± 3.04^{b}
MEO	71.46 ± 2.52^{a}	72.75 ± 4.11^{a}	72.41 ± 4.21^{a}	72.31 ± 2.84^{a}	70.77 ± 2.72^{a}	68.75 ± 2.53^{a}
CH+MEO	69.60 ± 1.60^{a}	70.95 ± 3.25^{a}	71.66 ± 4.53^{a}	71.32 ± 4.80^{a}	68.56 ± 4.44^{a}	63.28 ± 3.4^{b}
			b* value			
Control	68.68 ± 2.14^{a}	67.39 ± 3.43^{a}	66.09 ± 3.40^{a}	63.55 ± 0.62^{a}	62.31 ± 2.74^{a}	62.04 ± 0.45^{a}
CH	68.38 ± 1.81^{a}	66.80 ± 0.84^{a}	65.69 ± 2.25^{a}	64.42 ± 1.84^{a}	62.58 ± 1.68^{a}	61.75 ± 3.17^{a}
MEO	67.98 ± 2.06^{a}	67.29 ± 2.50^{a}	65.97 ± 3.42^{a}	65.53 ± 1.16^{a}	63.88 ± 3.65^{a}	63.43 ± 2.21^{a}
CH+MEO	68.11 ± 0.81^{a}	66.03 ± 1.32^{a}	65.72 ± 3.04^{a}	65.33 ± 3.36^{a}	62.23 ± 2.49^{a}	62.33 ± 1.54^{a}
			Firmness (N)			
Control	17.74 ± 1.57^{a}	13.20 ± 2.46^{a}	9.90 ± 2.35^{b}	10.66 ± 3.29^{b}	11.30 ± 1.15^{b}	11.42 ± 1.16^{a}
CH	17.55 ± 1.85^{a}	11.90 ± 0.40^{a}	12.15 ± 0.92^{ab}	12.06 ± 2.11^{ab}	10.52 ± 2.49^{b}	9.66 ± 2.33^{a}
MEO	16.36 ± 2.12^{a}	15.11 ± 3.42^{a}	13.91 ± 1.62^{a}	15.32 ± 2.59^{a}	15.94 ± 2.04^{a}	11.39 ± 1.76^{a}
CH+MEO	15.19 ± 3.28^{a}	12.38 ± 1.37^{a}	11.42 ± 0.36^{ab}	12.18 ± 2.53^{ab}	9.36 ± 3.25^{b}	10.41 ± 2.54^{a}
			TSS (°Brix)			
Control	15.22 ± 0.61^{a}	15.91 ± 0.56^{a}	15.97 ± 0.32^{a}	16.97 ± 0.54^{a}	17.13 ± 0.45^{a}	16.93 ± 0.76^{a}
CH	14.64 ± 0.45^{a}	15.33 ± 0.41^{ab}	16.13 ± 0.39^{a}	16.42 ± 0.39^{a}	16.80 ± 0.31^{a}	17.04 ± 0.11^{a}
MEO	15.02 ± 0.50^{a}	15.15 ± 0.89^{b}	16.40 ± 0.51^{a}	16.30 ± 0.33^{a}	16.48 ± 0.54^{a}	16.48 ± 0.74^{a}
CH+MEO	15.31 ± 0.96^{a}	15.66 ± 0.76^{ab}	16.44 ± 0.41^{a}	16.53 ± 0.41^{a}	17.02 ± 0.62^{a}	16.86 ± 0.28^{a}
			TA			
Control	0.80 ± 0.08^{a}	0.79 ± 0.11^{a}	0.79 ± 0.06^{a}	0.66 ± 0.05^{a}	0.57 ± 0.05^{a}	0.52 ± 0.09^{a}
CH	0.78 ± 0.02^{a}	0.77 ± 0.11^{a}	0.76 ± 0.06^{a}	0.68 ± 0.07^{a}	0.59 ± 0.06^{a}	0.57 ± 0.09^{a}
MEO	0.72 ± 0.04^{a}	0.76 ± 0.09^{a}	0.79 ± 0.03^{a}	0.61 ± 0.07^{a}	0.52 ± 0.06^{a}	0.50 ± 0.05^{a}
CH+MEO	0.74 ± 0.02^{a}	0.75 ± 0.07^{a}	0.72 ± 0.08^{a}	0.65 ± 0.16^{a}	0.58 ± 0.09^{a}	0.57 ± 0.08^{a}

Note: for each parameter, different letters on the same evaluation day indicate a significant difference among treatments. Fisher's Least Significant Difference (LSD) test ($p \leq 0.05$).

cherry tomato fruit, where a lower weight loss was registered in fruit coated with *Origanum vulgare* essential oil (1.25 µL/mL) during 12 days of storage at 25 °C or 24 days at 12 °C. For their part, Xylia *et al*. (2021) reported that the application of marjoram essential oil, chitosan and their combination did not affect the lightness of fresh-cut lettuce stored for 6 days.

With respect to b* parameter, only the storage time factor had a significant effect ($p \leq 0.05$) (Table 1). At the beginning of the storage, the mango slices had b* values of about 68, which gradually decreased during the storage reaching values of about 62-64 for all treatments; due to the b* value represents the blue–yellow components, with negative numbers toward blue and positive toward yellow, this decrease in b* value represents a decrease in the yellow coloration of the slices. No significant differences were shown among treatments during storage which is similar to the results observed by Picard *et al*. (2013), who reported that there were no significant differences in internal and external color of whole papayas treated with chitosan (1 %), peppermint essential oil (0.2 %) and their combination respect to the untreated papayas. Also, b* value in fresh-cut lettuce stored for 6 days at 7°C decreased by the application of pure chitosan, while marjoram essential oil and its combination did not present significant differences with respect to the control (Xylia *et al*., 2021). Nevertheless, Sarengaowa *et al*. (2022) informed that color of fresh-cut potatoes treated with chitosan and cinnamon essential oil depends on the amount of essential oil used, since the appearance of fresh-cut potatoes treated with chitosan and 0.2 % of cinnamon essential oil was better than those untreated; however, potato treated with chitosan and cinnamon essential oil at 0.4 % or 0.6 % had a negative effect, which was attributed to a phytotoxic effect of the essential oil when used at high concentrations.

The firmness of mango slices was affected by storage time and the applied treatments ($p \leq 0.05$). Overall, the firmness values decreased with the storage time; the highest firmness was observed in slices treated with MEO, which remained without significant changes during the first 12 days of the storage; after 15 days, a decrease was observed, and no significant differences were shown between treatments. CH and CH+MEO treatments did not show significant effect upon firmness during storage at 5 °C.

It was expected that fruit treated with CH alone and combined with MEO would have greater firmness than untreated fruit as reported by previous authors in different fresh-cut fruit such as pineapple, green beans, potatoes, and guavas (Minh *et a*l., 2019; Abdelgawad *et al*., 2022; Sarengaowa *et al*., 2022; Hanani *et al*., 2023), however, only fruit treated with MEO had higher firmness than the control group. Abdelgawad *et al*. (2022) attributed the positive role of the essential oil for maintaining firmness to their antimicrobial properties, due to less degradation of pectin caused by microbiological spoilage. Similar results were reported by Picard *et al*. (2013) who found that the application of chitosan, peppermint essential oil or the combination chitosan plus peppermint essential oil did not affect the firmness of papaya fruit as compared to the control fruit.

Total soluble solids and titratable acidity in fresh-cut mango

The fresh-cut fruit had an initial TSS average value of 15 °Brix, which increased during storage for all treatments, reaching values of approximately 16.5 °Brix at the end of storage. Only at day 3, MEO exhibited significant difference with control, where the mango slices treated with MEO presented lower TSS values; the rest of the storage, no significant differences were presented among treatments. Similar results were reported by Xylia *et al*. (2021) who observed that the application of chitosan, marjoram essential oil and their combination did not affect the TSS content of fresh-cut lettuce. Also, Picard *et al*. (2013) did not find significant differences between untreated papayas and papayas treated with chitosan, peppermint essential oil and the combination chitosan plus peppermint essential oil.

The TA of fresh-cut mango was affected by storage time ($p \leq 0.05$) but not by treatment application. A decrease was observed in all treatments as the storage time progressed, starting with citric acid values of 0.72 %-0.80 % at the time of processing and reaching values of 0.50 %-0.59 % of citric acid at the end of storage. Bitencourt *et al*. (2014) reported similar results in fresh-cut pineapple treated with a dimethyl sulfoxide-based edible coating incorporated with MEO, where TA was not affected by the treatment. They mention that higher TA values are preferred in fresh fruit, because they help to prevent microbial development. Contrary to our results, Hanani *et al*. (2023) observed that fresh-cut guava coated with chitosan and cinnamon essential oil exhibited a greater TA than the untreated ones. The authors discussed that chitosan act as a barrier to reduce respiration and ripening, thus preserving fruit quality. TA is an indicator of the amount of acids present, and during ripening, organic acids are broken down, which reduces acidity. By slowing down ripening through the reduction of respiration, this acid breakdown is retarded, helping to maintain TA at higher levels, preserving longer fruit quality. In a study reported by Xylia *et al*. (2021), only the application of marjoram essential oil had effect on TA since an increase was observed in fresh-cut lettuce treated with this oil compared with untreated, and the application of chitosan and the combination had no significant effect.

Some reported investigations show that chitosan alone or combined with essential oil has had an effect in delaying the ripening process, because they retard changes in color, firmness, TSS, and TA in whole fruits such as peach and in fresh-cut fruits such as pineapple and green beans (Minh *et al*., 2019; Rahimi *et al*., 2019; Abdelgawad *et al*., 2022). Despite this, in the present study neither the individual treatments (CH and MEO) nor the combined treatment (CH+MEO) negatively impacted the quality of fresh-cut mango, since they did not show differences with the untreated mango slices.

Bioactive compounds content

Ascorbic acid, total phenolics, and β-carotene contents are shown in Figure 2. At the beginning of the storage, fresh-cut mango had an average ascorbic acid content of 0.45 mg of

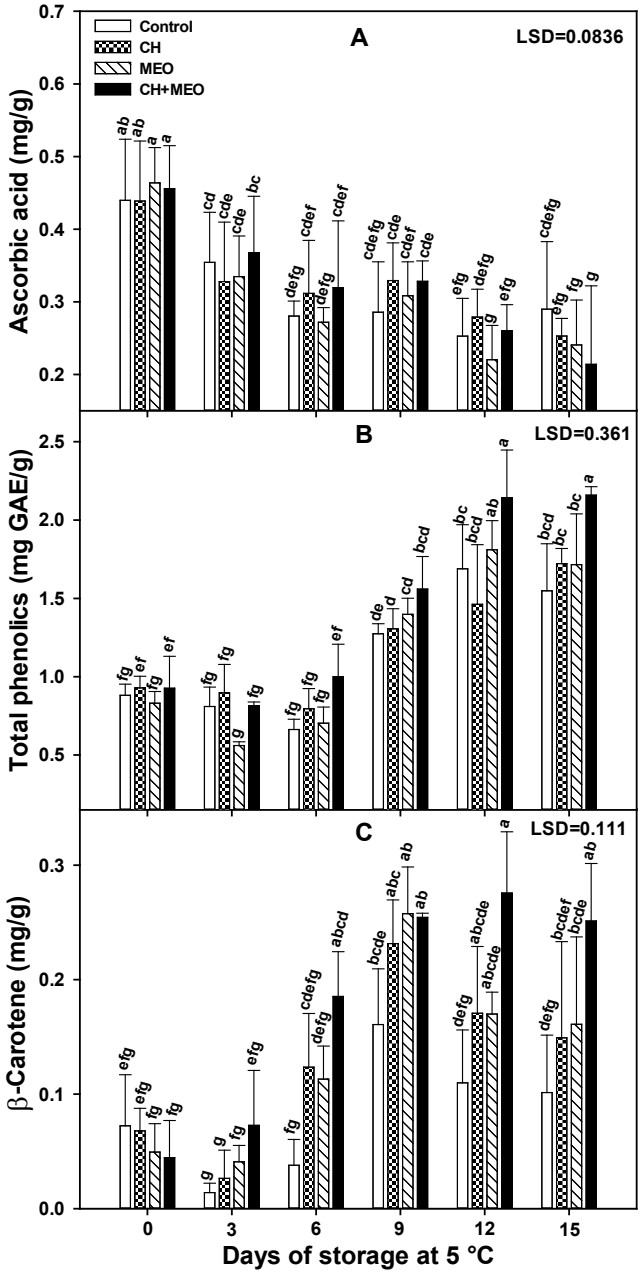

Figure 2. Effect of chitosan (CH), mint essential oil (MEO), and the combination (CH+MEO) on ascorbic acid content (A), total phenolics (B), and β-carotene (C) of mango slices stored during 15 days at 5 °C. Vertical bars on columns represent standard deviation of the mean. Different letters indicate significant differences among treatments and days. Fisher's Least Significant Difference (LSD) test ($p \leq 0.05$).

Figura 2. Efecto del quitosano (CH), aceite esencial de menta (MEO) o su combinación (CH+MEO) sobre el contenido de ácido ascórbico (A), fenoles totales (B) y β-caroteno (C) de mango fresco cortado almacenado durante 15 días a 5 °C. Las barras verticales en las columnas representan la desviación estándar de las medias de tres réplicas. Las letras diferentes indican diferencias significativas entre tratamientos y días.

ascorbic acid/g FW; this content decreased at the end of the storage for all treatments, reaching values of 0.22, 0.24, 0.26, and 0.30 mg of ascorbic acid/g FW in CH+MEO, MEO, CH, and control, respectively (Figure 2A). It is worth mentioning that there were no significant differences among treatments on any evaluation day. The results obtained in this study coincide with those found by Xylia *et al*. (2021), since the application of marjoram essential oil, chitosan, and their combination did not have any effect on the ascorbic acid content on fresh-cut lettuce. However, the results are different to those reported by Alikhani (2014), who reported that fresh-cut mango coated with rosemary oil and opuntia mucilage-rosemary oil had higher ascorbic acid content than the uncoated one during 9 days of storage at 6 °C. Likewise, Abdelgawad *et al*. (2022) reported that ascorbic acid content decreased during the storage of fresh-cut green beans, and the application of CH y CH+MEO treatments showed significantly higher ascorbic acid content compared to the control. Also, Xing *et al*. (2015) observed a synergistic effect when 1 % chitosan was combined with 0.1 % cinnamon oil, because despite the fact that the application of the individual treatments contributed to increase the content of ascorbic acid in jujube fruit, the combination presented the highest content; they attributed their results to a lower ripening rate because chitosan could reduce oxygen content and the loss of ascorbic acid can be favored by the presence of oxygen; also, cinnamon oil could act as antibrowning agent. In our results, a synergistic effect of chitosan and mint essential oil was not observed, which could be attributed to the fact that the concentration used of essential oil was 5 times lower than that used in the study of Xing *et al*. (2015).

With respect to total phenolics, the initial content was about 0.90 mg GAE/g FW, which remained constant during the first 6 days of storage in the four treatments; however, an increase was observed during the rest of the storage, showing significant differences among treatments from day 12, where CH+MEO had the highest phenolic content and control, while CH and MEO treated slices had no significant differences among them (Figure 2B). The preservation of total phenolics compounds in whole and fresh-cut fruits treated with chitosan and essential oils has been reported by other researches, as the work published by Abdelgawad *et al*. (2022), where total phenolic content was higher in fresh-cut green beans treated with chitosan plus tea tree oil, or chitosan plus peppermint oil, than in untreated ones or those treated with chitosan. Xing *et al*. (2015) observed an accumulation of total phenolics in jujube fruit treated with chitosan, cinnamon oil, and chitosan-cinnamon oil, except for the control fruit, having the highest values in fruit treated with the combination. In the present study, the highest phenolics content observed in treated mango slices can be attributed to plant defense activation by chitosan (Xing *et al*., 2015). Also, the essential oil could act by slowing or preventing the oxidation of phenolics due to their reaction with free radicals and also serving as oxygen scavengers (Xing *et al*., 2015). According to Barreto *et al*. (2016), a coating of chitosan-essential oil can affect the metabolism of phenolics inducing the modification of their internal atmosphere causing the synthesis and accumulation of these compounds.

β-carotene content was affected by both factors, storage time and applied treatments (Figure 2C). Concerning

the storage time, an increase was observed in CH, MEO and CH+MEO as the time progressed, starting from values of about 0.05 mg β-carotene/g FW and reaching a value 5 times higher at the end of the storage for slices treated with the combination CH+MEO. On the applied treatments, it was not observed differences among treatments during the first three storage days; however, from day 6, control slices had the lowest β-carotene content, followed by CH and MEO, while CH+MEO treated slices had the highest values.

The higher β-carotene values observed in this study by CH+MEO can be attributed to increased protection conferred by the coating, since carotenoids are compounds highly susceptible to oxidation and isomerization, which causes its content to decrease (Ayón-Reyna *et al*., 2015). Similar results were reported in peach fruit, where fruit treated with the chitosan-thymol essential oil combination had the highest carotenoid content, followed by the individual treatments, chitosan and thymol essential oil, while control fruit had the lowest content (Rahimi *et al*., 2019). Contrary to our results, Xylia *et al*. (2021) reported a decrease in total carotenoids in fresh-cut lettuce treated with chitosan or marjoram essential oil.

Antioxidant capacity

The lowest initial antioxidant capacity value was observed in the control treatment, followed by MEO, CH+MEO, and CH (Figure 3). The antioxidant capacity increased for CH and CH+MEO treatments; however, control and MEO treatments remained without significant changes for the rest of the storage. At day 15, significant differences were observed between control and the rest of the treatments, where control slices had the lowest values (0.14 µmol TE/g) followed by MEO (0.23 µmol TE/g); also, CH and CH+MEO treated slices had the highest values (about 0.4 µmol TE/g) and no significant differences were observed between them.

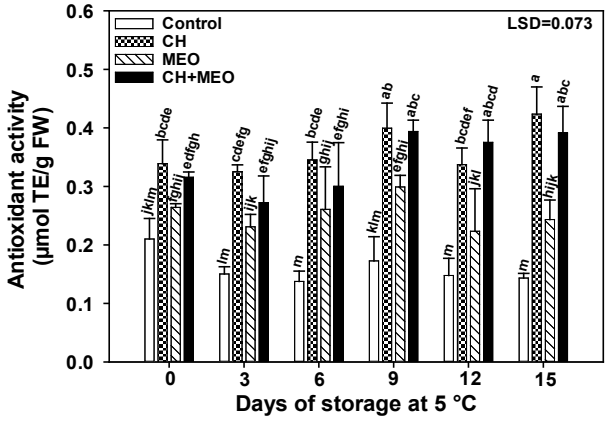

Figure 3. Changes in antioxidant capacity measured by DPPH method during the storage of mango slices coated with chitosan (CH), mint essential oil (MEO), and the combination (CH+MEO). Vertical bars on columns represent standard deviation of the mean. Different letters indicate significant differences among treatments and days. Fisher's Least Significant Difference (LSD) test ($p \leq 0.05$).

Figura 3. Cambios en la capacidad antioxidante medida por el método DPPH durante el almacenamiento de mango fresco recubierto con quitosano (CH), aceite esencial de menta (MEO) o su combinación (CH+MEO). Las barras verticales en las columnas representan la desviación estándar de las medias de tres réplicas. Las letras diferentes indican diferencias significativas entre tratamientos y días.

These results are in concordance with the results found in fresh-cut green beans treated with chitosan, chitosan-tea tree oil, or chitosan-peppermint oil (Abdelgawad *et al*., 2022). It was also observed that a chitosan coating combined with ginger essential oils applied in fresh-cut cantaloupe was effective to increase the total antioxidant capacity (Chen *et al*., 2022). Nevertheless, our results do not agree with those reported by Xylia *et al*. (2019) who observed a decrease in antioxidant capacity of fresh-cut lettuce treated with chitosan and chitosan+marjoram essential oil. Due to the fact that CH and CH+MEO treatments did not present significant differences, a synergistic effect of these treatments cannot be accredited, so the highest content of antioxidant capacity observed in the combination is mainly attributed to chitosan. Chitosan is a biopolymer that exhibits antioxidant capacity due to its property of neutralizing reactive oxygen species, such as hydrogen peroxide, superoxide anion radicals and free hydroxyl radicals. Moreover, chitosan can increase the activity of enzymes directly related to the neutralization of reactive oxygen species, as peroxidases, superoxide dismutase and catalase. In addition to this, it interferes in the metal chelation, adsorption, and ion exchange because it takes part in interactions of amine groups with metal ions (Stasińska-Jakubas and Hawrylak-Nowak, 2022).

CONCLUSIONS

All treatments were effective to decrease the microbial growth and none of them affected the color development, firmness, SST, TA, and ascorbic acid of fresh-cut mango. The application of CH+MEO produced the largest increase in total phenolics and β-carotene content, while the highest antioxidant capacity was obtained in fruit treated with CH and CH+MEO. These results showed that this last coating has potential to be used in fresh-cut mango as an edible bioactive coating.

CONFLICTS OF INTEREST

The authors declared that they have no conflict of interest.

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