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Wet steam method for reducing attached cells of *Salmonella* and *Listeria monocytogenes* on Hass avocado ☆

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A wet steam treatment to improve the microbial safety of Hass avocado

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ABSTRACT

The consumption of avocados and their products has been linked to outbreaks of illness caused by *Salmonella enterica* and *Listeria monocytogenes*. These pathogens have been isolated from avocados collected from farms and markets. After contact with the avocado epicarp, the cells of *Salmonella* and *L. monocytogenes* can become loosely attached (LA) by suspension in a film of water and attraction by electrostatic forces, or strongly attached (SA) by physical and irreversible attachment mechanisms. Attached cells may have greater resistance to agents used to decontaminate the fruit. The effect of applying wet steam (WS) to the epicarp of Hass avocados on the reduction LA and SA counts of *Salmonella* and *L. monocytogenes* was evaluated as a function of the exposure time. The inoculated avocados were washed and exposed to WS for 30, 45, and 60 s inside a treatment chamber. *Salmonella* was found to be more susceptible to WS than *L. monocytogenes*. The efficacy of steam in reducing LA and SA cell numbers was similar for both pathogens. Steaming avocados for 60 s reduced LA *Salmonella* and *L. monocytogenes* cells by 4.6 and 4.8 log CFU/avocado, whereas SA cells were decreased by 5.2 and 4.4 log CFU/avocado, respectively.

- Steaming the avocados for 60 s produced the greatest reduction in loosely and strongly attached cells for both pathogens.
- Wet steam treatment efficiently eliminated the loosely and strongly attached cells of both pathogens.
- The *Listeria monocytogenes* attached cells showed greater resistance to steam treatment than *Salmonella*.

☆ **Related research article:** García-Frutos, R., Martínez-Gonzáles, N. E, Martínez-Chávez, L., Gutiérrez-González, P., Moscoso-Sánchez, F. J., Macías-Rodríguez, M. E. Effect of wet steam on the survival of *Salmonella* and *Listeria monocytogenes* cells attached to Hass whole avocado, LWT, Volume 184, 2023, 115,071, https://doi.org/10.1016/j.lwt.2023.115071.
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Method details

Materials and methods

Bacterial strains

Six Rifampicin-resistant (*R*+) mutants [1] derived from parent strains of *Salmonella* and six strains of *Listeria monocytogenes* were used to inoculate avocados (Table 1). The *Salmonella* strains consisted of five *Salmonella enterica* subsp. *enterica* strains corresponding to the serovars Typhimurium, Poona, Bardo (one strain each), and Bareilly (two strains) originally isolated from produce environments and avocados, which were obtained from the Food Microbiology and Safety Laboratory culture collection at the University of Guadalajara (FMSL-UDG). In addition, a strain of *S.* Typhimurium isolated from tomatoes, was acquired from the Laboratory for the Evaluation and Control of Microorganisms in Food at the Autonomous University of Queretaro (ECMF-UAQ). *Listeria monocytogenes,* five strains isolated from farm soil, avocados, and a clinical sample were obtained from the FMSL-UDG collection, whereas the sixth strain was provided by ECMF-UAQ, which was originally isolated from commercial guacamole. Cultures of each strain were stored in tryptic soy broth (TSB; Bioxon, Becton Dickinson, U.S.A.) supplemented with 20 % glycerol at −80 °C for long-term preservation. Working cultures of both pathogens were preserved on tryptic soy agar (Bioxon, Becton Dickinson, U.S.A.) supplemented with 0.6 % yeast extract (Bioxon, Becton Dickinson, U.S.A.) (TSAYE) slants at 5 °C for up to 30 days before the experiments.

Bacterial inoculum preparation and avocado inoculation

Fresh avocados (*Persea americana* var. Hass) were purchased from a local distribution center in Guadalajara, Mexico, ensuring all fruits were of the same origin, and transported to the FMSL-UDG at room temperature (25 ± 5 °C) for experimental studies on the next day. Avocados were unripe, unwaxed, unwashed, free of visual defects such as bruises, cuts, or abrasions, and were of a similar weight (200 ± 50 g). No washing or decontamination treatments were applied before inoculation.

Inoculum preparation and inoculation of avocados performed as described by García-Frutos et al. [2]. Briefly, *Salmonella* and *L. monocytogenes* strains were grown individually by transferring a loopful of each working culture from a TSAYE slant into 10 mL of TSB for *Salmonella* or TSB supplemented with 0.6 % yeast extract (TSBYE) for *L. monocytogenes,* then incubating at 35 °C for 18 h. After incubation, 100 µL from each culture was individually transferred to 10 mL of TSB or TSBYE and incubated at 35 °C for 6 h. Subsequently, 100 µL of this culture was transferred to 35 mL of TSB or TSBYE and incubated at 35 °C for 18 h. These cultures were washed twice using centrifugation (Durafuge 300R, Precision, Winchester, Virginia, U.S.A.) at 4507 × *g* and 5 °C for 10 min. The supernatant was discarded, and the pellet was resuspended in 35 mL of sterile saline solution (SS, 0.85 % NaCl). For each pathogen, the washed cell suspensions of the six strains were mixed manually in a sterile 710-mL Whirl-Pak® bag (Nasco, Wisconsin, U.S.A.) for 30 s to prepare two cocktails with 210 mL of each pathogen. The bacterial concentration in each cocktail was determined by preparing decimal dilutions in 0.1 % peptone water (PW) and surface-plating onto TSA supplemented with 100 µg/mL rifampicin (Sigma-Aldrich, St. Louis, U.S.A.) (TSA-Rif) for *Salmonella* and TSAYE-Rif for *L. monocytogenes,* incubating at 35 °C for 24 and 48 h, respectively, prior to counting colonies. These counts were determined to be 8.4 ± 0.2 log CFU/mL for *Salmonella* and 8.7 ± 0.2 log CFU/mL for *L. monocytogenes*.

Table 1
Salmonella enterica and *Listeria monocytogenes* strains used in this study.

Bacterium	Strain (ID)	Source
S. Typhimurium	Sm-R-035	Tomato epicarp
S. Typhimurium	Sm-R-037	Human feces, clinical case
S. Ponna	Sm-R-041	Avocado epicarp collected from avocado farms
S. Bardo	Sm-R-048	Compost from avocado farms
S. Bareilly	Sm-R-559	Avocado epicarp collected from local markets
S. Bareilly	Sm-R-743	Avocado epicarp collected from local markets
L. monocytogenes	Lm-R-018	Agricultural soil from avocado farms
L. monocytogenes Scott A, serotype 4b	ATCC 15,313	Human feces, clinical case
L. monocytogenes	N4	Commercial guacamole
L. monocytogenes	Lm-R-251	Avocado epicarp collected from local markets
L. monocytogenes	Lm-R-336	Avocado epicarp collected from local markets
L. monocytogenes	Lm-R-357	Avocado epicarp collected from local markets

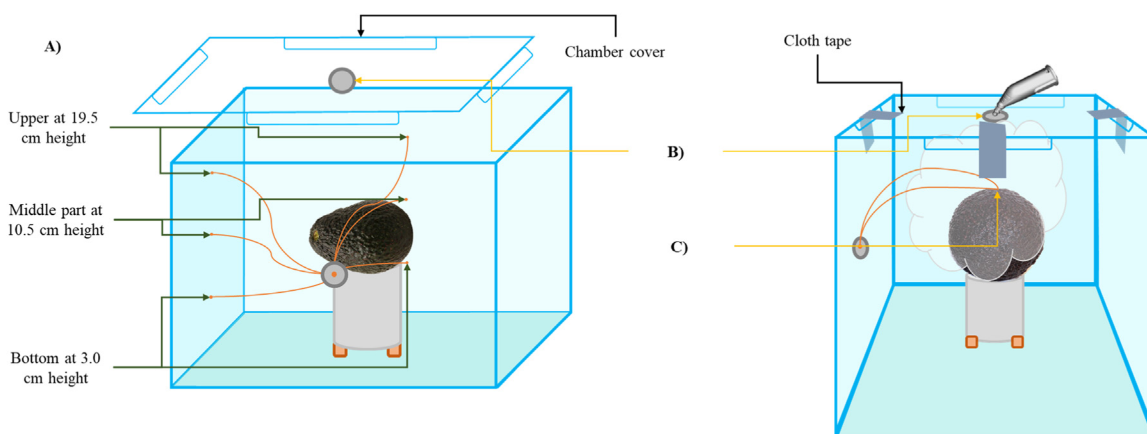

Fig. 1. Schematic diagram of the interior of the treatment chamber showing the position of wet steam application on the avocado surface and the places where the temperature was measured inside the chamber and on the fruit: (A) Position and distance of thermocouples inside the chamber, (B) Steam application, and (C) Avocado areas where thermocouples were inserted.

Whole avocados were superficially inoculated with *Salmonella* or *L. monocytogenes* cocktails to test the efficacy of steam decontamination. Experiments were conducted separately for each pathogen. Each avocado was immersed in the corresponding bacterial cocktail and manually shaken for 1 min to ensure a uniform inoculation. The avocados were then aseptically removed from the inoculum and placed on a stainless-steel stand previously sanitized with 70 % ethanol to drain and dry in a biosafety cabinet (Telstar, Bio II Advance Plus, Pennsylvania, U.S.A.) at room temperature (25 ± 5 °C) for 1 h. Two inoculated fruits were used to determine the initial LA and SA cell counts for both pathogens on the avocado epicarp, as described in the enumeration of attached cells section below. The total count (LA + SA) was obtained from the sum of the LA and SA cells. The counts of LA + SA, LA, and SA cells for *Salmonella* were 6.8 ± 0.5, 7.0 ± 0.6, and 5.9 ± 0.2 log CFU/avocado and those for *L. monocytogenes* were 7.4 ± 0.2, 7.3 ± 0.3 and 6.3 ± 0.3 log CFU/avocado, respectively. The remaining inoculated avocados were placed 2 cm apart from each other in sanitized polyethylene baskets (50 cm long × 30 cm wide × 6 cm height) and stored in a climatic chamber (Sheldon Manufacturing, model HC5RL, Cornelius, U.S.A.) at 25.4 ± 1.7 °C and 76.5 ± 4.7 % RH for 72 h to promote fruit ripening and the attachment of the pathogenic bacteria [3]. Temperature and RH were measured during storage of the avocados using a digital thermohygrometer (Traceable®, Webster, model 4096 CC, Texas, U.S.A.). After storage, two avocados were randomly selected for LA and SA cell enumeration of both pathogens and used as positive controls. The remaining avocados were used for steam decontamination. Four independent experiments were conducted, each with two replicates per treatment (n = 8).

Application of a method with wet steam to remove attached cells of pathogenic bacteria on the avocado epicarp

Before the WS application, all avocados were individually water-washed (WW) and placed in a polyethylene crate inside a Nalgene™ autoclavable polypropylene tub. The water wash consisted of spraying the avocados with 250 mL of chlorinated (20 mg/L) tap water for 15 s using an automatically compressed air sprayer constructed of non-corrosive polyethylene with a two-stroke engine (Kawashima, model ATMT26, P.R.C.). Each avocado was continuously rotated during the water wash, simulating avocado movement on a conveyor belt during washing, and was then placed on a stainless-steel stand at room temperature (25 ± 5 °C) to drain for 15 min. Two WW avocados were selected for LA and SA cell enumeration.

A VAPOHydro DE 4002 pressurized steam generator (Kärcher, Baden-Wurtemberg, Germany) was used for WS treatment, which supplies steam at 82 g/min and 100 °C. The boiler and generator reserve tanks are filled each with 2.2 L of tap water. The equipment was turned on for 18 min to generate steam. Subsequently, steam was expelled in a container for 20 s to generate a constant flow. A rectangular polyethylene chamber (31.5 cm long × 22.5 cm wide × 22.5 cm height) (Alica, model A344-12, Guadalajara, Mexico) with hermetic closure was employed for WS treatment (Fig. 1). Washed avocados were placed horizontally on a stainless-steel cylindrical stand (8 cm height, 5 cm diameter) inside the treatment chamber with the peduncle scar placed on the left side. The stand was raised 1 cm above the chamber base with four rubber plugs to allow steam flow (Fig. 1). The chamber was closed for WS application by placing cloth tape on the four sides of the lid to maintain an airtight seal during treatment (Fig. 1B). The nozzle of the generator was placed at the top central part of the chamber, 7 cm from the fruit. WS was applied to the avocados for 30, 45, or 60 s. The counts of LA and SA cells of *Salmonella* and *L. monocytogenes* on the treated avocado epicarps were then determined. Four independent experiments were conducted, each with two replicates per treatment (n = 8). After each experiment, the steam generator was washed with CLR all-purpose cleaner (Jelmar, Illinois, U.S.A.), according to the manufacturer's instructions, to remove salt deposits generated by the tap water.

Enumeration of attached cells

Each avocado was rinsed by placing in a Whirl-Pak® bag with 100 mL of buffered peptone water (BPW, DIFCO Becton Dickinson and Company, Sparks, MD, U.S.A.) and manually shaking at a 90° angle for 20 s to recover the LA cells of *Salmonella* and *L. monocytogenes*. Subsequently, the avocado was transferred to another sterile bag with 100 mL BPW and sonicated in an ultrasound bath (Fisher Scientific, model 15,337,418, U.S. A.) at 300 W and 40 kHz for 1 min to detach the SA cells from both pathogens, according to the methodology of Martínez-Chávez [3].

For untreated samples, WW, and WW + WS for 30, 45, and 60 s, LA and SA cells were enumerated by preparing dilutions in PW of the rinse and sonication liquids, and surface-spreading 0.33 mL of each dilution on triplicate plates of TSA-Rif or TSAYE-Rif plates to enumerate *Salmonella* and *L. monocytogenes*, respectively. The plates were incubated at 35 °C for 24 h for *Salmonella* and 48 h for *L. monocytogenes*. The membrane filtration method was also used to enumerate LA and SA cells in avocados treated with WW + WS for 60 s to increase the sensitivity of the methods. From each rinse liquid of LA and SA cells, 50 mL was filtered through a 0.45 µm nitrocellulose membrane (Sartorius Stedim Biotech, Göttingen, Germany) using vacuum filtration equipment (Millipore, Sigma, Burlington, MA, U.S.A.). The membranes were placed on TSA-Rif for *Salmonella* and TSAYE-Rif for *L. monocytogenes* plates and incubated at 35 °C for 24 or 48 h, respectively. After incubation, a membrane filter was used to count the colonies of both pathogens. Gram staining and biochemical tests were conducted to confirm the identity of each pathogen. The biochemical tests for *Salmonella* include lactose/glucose fermentation + H_2S production in triple sugar iron agar, lysine decarboxylase + H_2S in lysine iron agar, and urease in urea broth [4]. For *L. monocytogenes* biochemical tests included fermentation of xylose, rhamnose, and mannitol [5].

Determination of the temperature profile on the epicarp and pulp of the avocado, as well as inside the chamber during steam application

Fig. 1A–C shows the temperature measurement scheme. To measure the temperature of the avocado epicarp and pulp during WS application, two type K thermocouples (Thomas Scientific, Swedesboro, U.S.A.) connected to a digital thermometer (Amprobe, TMD-56, Seattle, U.S.A.) were inserted into the equatorial zone of the non-inoculated whole avocado (Fig. 1C). An incision no more than 1 mm deep was made on the avocado rind with a scalpel blade to place a thermocouple and measure the temperature on the avocado epicarp, and a thermocouple inserted 4 mm deep was used to monitor the temperature in the avocado pulp. Thermocouples were attached to the avocado epicarp with cloth tape to prevent detachment during the WS treatment. Temperature data were recorded every 5 s for 60 s. The environmental temperature in the treatment chamber during the WS application was also measured by placing thermocouples at heights of 3, 10.5, and 19.5 cm at the center of the chamber (Fig. 1A). These thermocouples were secured to the walls of the chamber using cloth tape. Temperature data were recorded every 5 s for 60 s. The experiment was performed twice, each with three repetitions for a total of six measurements ($n = 6$). Photographs of fruits untreated and treated with WS were obtained to record the changes in the appearance of the avocado epicarp and pulp.

Data analysis

The experimental design was a randomized complete block design in which we evaluated the effect of each treatment on the counts of LA + SA, LA, and SA cells for each pathogen. The attached cell counts for each pathogen were transformed into log CFU/avocado values. Standard deviation (SD) was used to express the variability related to pathogen counts in the treatments. The counts of LA + SA, LA, and SA cells for each pathogen were compared using one-way analysis of variance (ANOVA) to measure the effects of the treatments. Furthermore, ANOVA was performed to compare the counts of LA or SA cells of both pathogens in each treatment to determine the effect according to the attached cell type. The least significant difference multiple range test was used to determine significant differences ($P < 0.05$) between the mean factor values. All data analyses were performed using StatGraphics Centurion 18 (Statpoint Technologies, Inc., Warrenton, VA, U.S.A.).

Method validation

Efficacy of wet steam reducing *salmonella* and *L. monocytogenes* cells attached on the avocados epicarp

The Hass avocado generally consumed fresh in several dishes and drinks. Outbreaks of salmonellosis and listeriosis associated with the consumption of contaminated avocado and avocado products have been reported [6]. *Salmonella* and *L. monocytogenes* were isolated from whole Hass avocado samples collected at various stages of the supply chain [7,8]. These pathogenic bacteria can be present both as LA cells by electrostatic forces and as SA cells, which have established physical and irreversible attachments to the avocado epicarp and can form biofilms [2,9]. The use of treatments such as washing with chlorinated water or lactic acid solutions has been evaluated as a step to reduce the contamination of avocados with pathogenic bacteria. These treatments showed limited effectiveness in removing total counts, with reductions of < 2 log CFU/avocado [7]. Decontamination treatment effectively reduced LA and SA cells. The Hass avocado has a rough epicarp where bacterial cells can become trapped and favor their attachment, limiting the effectiveness of decontamination treatments. Physical agents such as WS can penetrate cracks, and when they condense, they promote quick heat transfer, effectively removing pathogens attached to rough surfaces. This study describes the application of WS treatment to reduce the populations of LA and SA cells of *Salmonella* and *L. monocytogenes* in the epicarp of ripe Hass avocados.

Table 2 shows the mean counts of attached cells of *Salmonella* and *L. monocytogenes* on the untreated Hass avocado epicarps (positive control), WW, and WW + WS for 30, 45, and 60 s. The magnitude of the reduction in the two pathogens varied with the

Table 2

Mean counts of attached cells of *Salmonella* and *Listeria monocytogenes* on the Hass avocados epicarp treated with wet steam, according to the attachment cell type and exposition time.

Treatment[a]	Mean counts[b] log CFU/avocado											
	Salmonella						*L. monocytogenes*					
	Total count		LA		SA		Total count		LA		SA	
	Mean ± SD	Minimum - Maximum	Mean ± SD	Minimum - Maximum	Mean ± DS	Minimum - Maximum	Mean ± SD	Minimum - Maximum	Mean ± SD	Minimum - Maximum	Mean ± DS	Minimum - Maximum
No treatment	6.7 ± 0.5 A	6.0–7.4	5.9 ± 0.8 Ax[c]	4.7–7.2	6.5 ± 0.6 Ax	5.5–7.1	6.6 ± 0.8 A	5.2–7.6	6.0 ± 1.0 Ax	4.7–7.6	6.3 ± 0.7 Ax	5.1–7.5
WW	4.8 ± 0.9 B	3.3–5.4	4.3 ± 0.8 Bx	3.0–5.1	4.6 ± 0.9 Bx	3.0–5.3	5.9 ± 0.9 A	4.5–7.1	5.6 ± 0.9 Ax	4.0–6.6	5.6 ± 1.0 Ax	4.3 –7.1
WW + WS 30 s	4.5 ± 1.2 B	2.7–5.7	4.1 ± 1.2 BCx	2.6–5.2	4.0 ± 1.3 Bx	1.7–5.5	5.9 ± 1.0 A	4.6–7.5	5.2 ± 1.3 Ax	3.1–7.0	5.7 ± 1.0 Ax	4.4–7.3
WW + WS 45 s	3.8 ± 1.5 B	1.0–5.2	3.1 ± 1.5 Cx	0.6–5.0	3.6 ± 1.5 Bx	0.8–4.7	4.1 ± 1.9 B	<0.3–5.9	3.2 ± 2.2 Bx	<0.3–5.2	4.0 ± 1.9 Bx	<0.3 - 5.8
WW + WS 60 s	1.6 ± 1.6 C	<0.3–4.5[d]	1.3 ± 1.4 Dx	<0.3–3.7	1.3 ± 1.6 Cx	<0.3–4.5	2.0 ± 2.0 C	<0.3–4.6	1.2 ± 1.4 Cx	<0.3–3.2	1.9 ± 2.0 Cx	<0.3–4.6

[a] No treatment = positive control; WW = spraying water for 15 s; WW + WS 30 *s* = WW followed by wet steam for 30 s; WW + WS 45 s = WW followed by wet steam for 45 s; WW + WS 60 s = WW followed by wet steam for 60 s.

[b] Values are means ± standard deviation of four trial experiments with two determinations for each treatment (n = 8). LA = loosely attached cells; SA = strongly attached cells, Total count = LA + SA cells; SD = standard deviation.

[c] Values within columns for each count with a different letter (A, B, C, D) are significantly different ($P < 0.05$). Values within rows for each LA and SA count with the same letter (x) are not significantly different ($P > 0.05$).

[d] The minimum detection limit was 2 CFU/avocado (0.3 log CFU/avocado). When no colonies were recovered from the treated avocado, < 0.3 log CFU/avocado was reported.

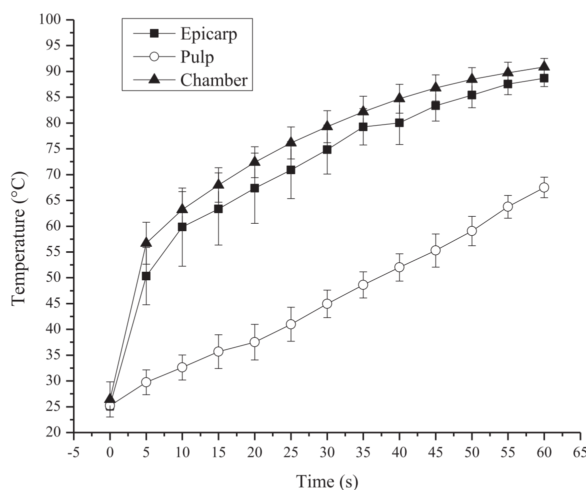

Fig. 2. Temperature profile of the average on avocado epicarp, pulp, and inside the treatment chamber during 60 s of wet steam application. Symbols represent the means (±SD) of six replications.

treatment applied to the avocado epicarp. When comparing LA and SA cell counts for each treatment, the type of cell attachment did not affect ($P > 0.05$) the removal of both pathogens. The mean LA + SA counts observed for *Salmonella* and *L. monocytogenes* on untreated avocados were 6.7 ± 0.5 and 6.6 ± 0.8 log CFU/avocado, respectively. In contrast, mean LA cell counts were 5.9 ± 0.8 and 6.0 ± 1.0 log CFU/avocado, whereas the mean counts of SA cells were 6.5 ± 0.6 and 6.3 ± 0.7 log CFU/avocado, respectively. After washing avocados, LA and SA levels of *Salmonella* were reduced by 1.6 and 1.9 log CFU/avocado and those of *L. monocytogenes* were reduced by 0.4 and 0.7 log CFU/avocado, respectively (Table 2).

The application of WS reduced the counts of *Salmonella* and *L. monocytogenes* on the avocado epicarp (Table 2). *Salmonella* spp. are generally more susceptible to WS than *L. monocytogenes*. Steam decontamination resulted in mean LA cell counts of 1.3–4.1 log CFU/avocado for *Salmonella* and 1.2–5.2 log CFU/avocado for *L. monocytogenes,* whereas SA cell counts were from 1.3 to 4.0 log CFU/avocado and 1.9–5.7 log CFU/avocado, respectively. No significant difference ($P > 0.05$) was observed between LA and SA *Salmonella* counts for fruits steamed for 30 or 45 s. For *L. monocytogenes*, the counts for both types of attached cells decreased ($P < 0.05$) as the duration of steam exposure increased. Steaming avocados for 60 s reduced *Salmonella* and *L. monocytogenes* counts by 4.6 and 4.8 log CFU/avocado for LA cells and 5.2 and 4.4 log CFU/avocado for SA cells, respectively. The exposure time to WS was a determining factor in achieving higher levels of pathogen reduction in avocados.

Fig. 2 shows the increase in temperature inside the chamber and in the avocado epicarp and pulp as the WS exposure time changed. The avocado epicarp and pulp temperatures at the beginning of the WS treatment (0 s) and after 60 s exposure were 25.3 and 84.6 °C and 25.0 and 61.9 °C, respectively. This indicated that the epicarp and the avocado pulp exhibited thermal resistance to heat flow. The greater increase in the epicarp temperature indicates that it acts as a thermal insulator of the pulp during exposure to steam. The ability of the epicarp to act as a thermal insulator has also been reported by Pao and Davis [10] during the immersion of Valencia oranges in hot water at 70 and 80 °C for 2 min.

The rapid heat transfer rate during the WS application for 30, 45, or 60 s changed the color and physical appearance of the avocado epicarp (Fig. 3B–D) but not of the avocado pulp (Fig. 3F–H). The color of the epicarp changed from purplish black (untreated avocado) to green, opaque, and then whitish after 30 s of WS exposure. Green and brown tones were observed after vaporizing for 45 s, and olive-green tones were observed after treatment for 60 s. Cuticle detachment was observed in some areas, and the pulp and the epicarp separated slightly after vaporizing for 60 s. These results show that the epicarp underwent higher modifications as contact time increased. According to our observations and the fruit pulp's palpable consistency, no damage was observed after WS treatment. Additional studies are necessary to evaluate the effects of WS treatment on the physicochemical quality of avocado fruits.

Experiments with inoculated avocados showed that the most significant reductions in LA + SA, LA, and SA *Salmonella* and *L. monocytogenes* were achieved after 60 s of exposure to WS, improving the safety of ripe Hass avocados. During WS application, the surrounding microdroplets transfer heat to the fruit more uniformly after 60 s than after 30 and 45 s [11]. However, when greater reductions in cells are achieved, higher temperatures were also reached, which could affect the quality of the avocado pulp. The results showed that WS treatment did not eliminate the cells attached to the avocado epicarp of either pathogen, probably because of the localization and attachment of the bacteria to crevices or extracellular polymeric substances that protect cells from thermal damage [2,9]. However, the significant reduction in the counts of LA and SA cells of *Salmonella* and *L. monocytogenes* highlight the potential effectiveness of WS in decontaminating the avocado epicarp. The reduced levels of both pathogens in steamed avocados resulted from the agent's exposure time, the sensitivity of the strains used in the inoculum, and the differences in epicarp roughness between the avocados, among other factors.

Previous studies have shown that steam is an alternative for inactivating *Salmonella* and *L. monocytogenes* in the epicarp of Valencia oranges, cherry tomatoes, and cantaloupes [12–14]. A comparison of our results with the findings of these studies should be made

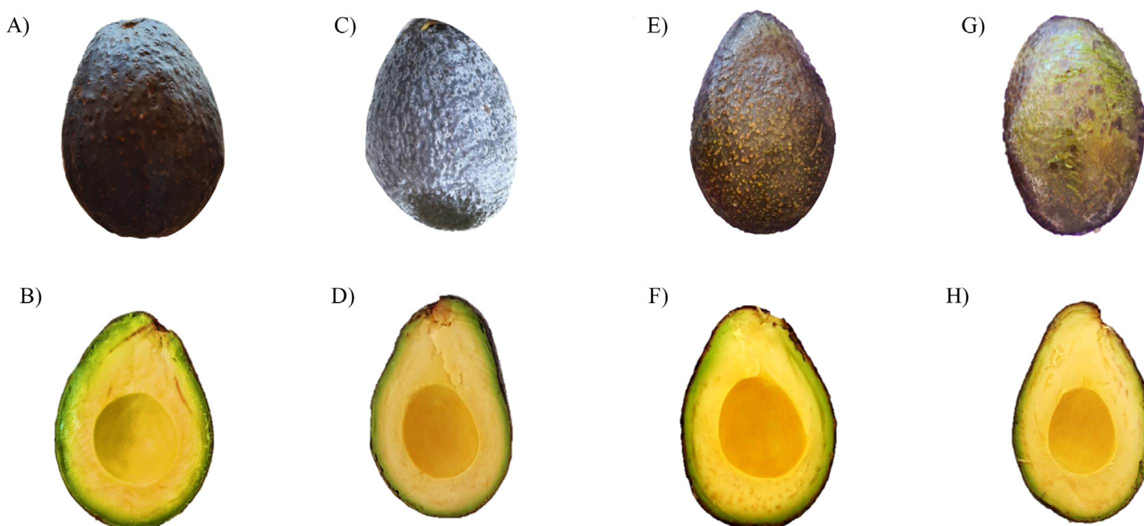

Fig. 3. Effect of steam treatments on the visual characteristics of avocados epicarp and pulp: untreated (A, B), treated with wet steam for 30 s (C, D), 45 s (E, F) and 60 s (G, H).

cautiously, owing to differences in fruit characteristics, ripening stage, inoculation procedure, application method, testing method, and reporting units, among other factors. It is also essential to consider that these studies reported total counts without differentiating between LA and SA cells. A study reported the effectiveness of saturated steam (SS) at 100 °C and superheated steam (SHS) at 200 °C to inactivate *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* in the epicarp of cherry tomatoes and Valencia oranges [12]. Ban and Kang [12] spot-inoculated 0.1 mL of each pathogen on the epicarp of tomatoes and oranges and then dried for 1 h at 22 ± 2 °C. Both types of fruits were subjected to steam treatments without being subjected to washing with water. After applying SS at 100 °C for 3 s on cherry tomatoes, attained log reductions of 1.4–2.7 for the three pathogens. In oranges, the application of SS at 100 °C for 20 s achieved log reductions of 2.3–2.6. Meanwhile, the SHS application at 200 °C for 3 s on cherry tomatoes and 20 s on oranges led the counts for the three pathogens to undetectable levels, < 1 log CFU/g for tomatoes and < 1.7 log CFU/g for oranges. Similar to the results reported by Ban and Kang [12] with the use of SS at 100 °C, we found similar reductions in *Salmonella* and *L. monocytogenes* in avocados treated with WS for 30 s, whereas steaming avocados for 60 s led to higher reductions in both pathogens. In contrast, SHS at 200 °C achieved reduction levels higher in *Salmonella* and *L. monocytogenes* in tomatoes and oranges epicarp than those we attained in avocados treated with WS. The main difference between this study and ours is that they utilized a process that involved heating SS at 200 °C to generate and supply SHS at constant pressure and temperature into the treatment chamber. The process required specialized equipment. Meanwhile, we employed a conventional homemade steam generator that supplies WS at 100 °C and can be purchased more easily by users.

Previous research published by Ukuku et al. [13] reported that applying WS at 100 °C for 180 s without moving the nozzle on the epicarp of cantaloupes reduced *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* counts by 4.4, 4.1 and 3.8 log cycles, respectively. Meanwhile, applying the WS with sweeping nozzle movements reduced the counts in 3.4, 3.0, and 3.3 log cycles, respectively. In this study, steam was applied to fruit in an open system, and lower reductions in foodborne pathogen counts in cantaloupes were achieved than in avocados treated with WS. Further, when interpreting this result, it must be considered that the steam was at the targeted spot, unlike in our study, where the steam diffused into the closed environment of the chamber. The main differences between the study of Ukuku et al. [13] and our study were a lower level of inoculum of pathogens used by them, lack of washing to cantaloupes before applying WS treatment, a higher distance between the nozzle and epicarp (7.6 cm, 68 °C cantaloupe epicarp vs. 7.0 cm, 84.6 °C avocado epicarp), and a longer exposure time (180 s cantaloupes vs. 60 s avocados). These studies demonstrate that WS treatment effectively removes pathogens from the cantaloupes and avocados' surface.

In another experiment conducted by Kim and Pao [14], the decontamination efficacy of three models of domestic electrical steamers (steamer-A, -B, and -C) for removing *Salmonella* and *L. monocytogenes* from cantaloupes was investigated. Fruits were spot inoculated with each pathogen and maintained at 4 or 22 °C for 24 h before applying steam treatment at 99 ± 1 °C. Their results showed that *Salmonella* was more susceptible than *L. monocytogenes* to steam treatment. The epicarp of cantaloupes maintained at 22 °C could be decontaminated (with ≥ 5.0 log reduction) within 60 s using any of the three steamers. However, for fruits stored at 4 °C, only steamers B and C effectively achieved these reductions in the counts. Similar to a report by Kim and Pao [14], we found a similar response of pathogens to WS treatment on avocados. However, the experimental design in both studies differed: spot inoculation of the cantaloupe epicarp vs. immersion inoculation of whole avocado epicarp. Also, unwashed cantaloupes were used to apply steam treatment, while washed avocados were employed to treat with WS. In addition, fruit ripeness at the time of inoculation time may influence the attachment of foodborne pathogens. In our study, green fruits were inoculated and incubated at 25 °C for 3

days to promote ripeness and attachment, which also favor biofilm formation [3]. In contrast, the experiments conducted by Kim and Pao [14] did not refer to the fruit ripeness level and were incubated at 4 and 22 °C for 24 h to promote attachment.

Conventionally, fresh fruits and vegetables are not subjected to heat treatment to improve food safety. Some heat treatments are used for phytosanitation or for quality purposes. The treatment of unripe Hass avocados with hot air at 40 or 38 °C for 0.5 and 3 h is applied to eliminate the larvae of the stone borer fly. The treatment also confers thermotolerance to the avocado, which prevents cold damage in the fruit, such as darkening or browning of the epicarp and pulp during refrigeration (0 to 6 °C) and prolongs the shelf life of the avocado [15]. Weller et al. [16] reported that applying WS at 38 and 40 °C for 8 h on Hass avocados eliminates insect larvae eggs, reduces cold damage to the fruits, and extends their shelf life. Steam treatment has not been used to obtaining pulp or other avocado products. However, the number of pathogenic microorganisms potentially present in the epicarp affects the safety of avocados and their products. It would be helpful to include effective decontamination treatments a these stages. The results of our study show the potential of WS to reduce *Salmonella* and *L. monocytogenes* LA and SA cell counts in the epicarp of ripe Hass avocado, with maximum effectiveness after 60 s of exposure. Steam application to reduce *Salmonella* and *L. monocytogenes* cells in the Hass avocado epicarp could be used in an integrated food safety program that applies good agricultural and hygiene practices during production and fruit management throughout the supply chain.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Ramón García-Frutos: Investigation, Writing – original draft, Methodology, Validation. **Nanci Edid Martínez-Gonzáles:** Resources, Methodology, Conceptualization, Writing – review & editing, Funding acquisition. **Liliana Martínez-Chávez:** Resources, Methodology, Conceptualization, Funding acquisition. **Porfirio Gutiérrez-González:** Formal analysis, Data curation. **Francisco Javier Moscoso-Sánchez:** Conceptualization, Writing – review & editing. **María Esther Macías-Rodríguez:** Conceptualization, Writing – review & editing.

Data availability

Data will be made available on request.

Ethics statements

N. A.

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