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Research Paper

Reduction Foodborne Pathogens and Surrogate Microorganism on Citrus Fruits after Lab- and Pilot-scale Finishing Wax Application

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ABSTRACT

After finishing waxes are applied, citrus fruits are typically dried at 32–60°C for 2–3 min before final packing. The survival of *Listeria monocytogenes*, *Salmonella*, and *Enterococcus faecium* NRRL B-2354 was evaluated under laboratory conditions on lemons after applying one of four finishing waxes (F4, F6, F8, and F15) followed by an ambient hold or heated (50 or 60°C) drying step. The reduction of inoculated microorganisms during drying was significantly influenced by wax type and temperature, with greater reductions at higher temperatures. Greater reductions after waxing and drying at 60°C were observed with *L. monocytogenes* (2.84–4.44 log) than with *Salmonella* (1.65–3.67 log), and with *Salmonella* than with *E. faecium* (0.99–2.93 log). The survival of *Salmonella* inoculated at 5.8–5.9 log/fruit on lemons and oranges after applying wax F6 and drying at 60°C was evaluated during storage at 4 and 22°C. The reductions of *Salmonella* after waxing and drying were 1.7 log; additional reductions during storage at 4 or 22°C were 1.40–1.43 or 0.18–0.29 log, respectively, on waxed lemons, and 0.56–1.02 or 0.54–0.57 log, respectively, on waxed oranges. Under pilot-scale packinghouse conditions with wax F4, mean and minimum reductions of *E. faecium* ranged from 2.15 to 2.89 and 1.64 to 2.12 log, respectively. However, *E. faecium* was recovered by whole-fruit enrichment (limit of detection: 0.60 log CFU/lemon) but not by plating (LOD: 1.3 log CFU/lemon) from uninoculated lemons run with or after the inoculated lemons. The findings should provide useful information to establish and implement packinghouse food safety plans.

The United States produced over 74,000 tons of citrus fruit in 2019 (Food and Agriculture Organization, 2021), with lemons and oranges being the most economically and agriculturally important. The application of waxes can effectively extend the shelf life of fresh citrus by suppressing the growth of plant pathogens that lead to spoilage and by preventing or reducing water loss and gas diffusion (Meighani et al., 2015; Ramírez et al., 2015; Singh et al., 2019; Tietel et al., 2010).

Foodborne illness outbreaks have not been associated with fresh citrus fruits but have been linked to the consumption of unpasteurized or reconstituted orange juice (Krug et al., 2020; McCollum et al., 2013; Parish, 1998). An outbreak strain, *Salmonella* Typhimurium phage type 135a, was found in fungicide and wax as well as on some waxed oranges during an investigation of a 1999 outbreak associated with Australian orange juice (Rajapakse, 2016). The primary source of contamination was determined to be the fungicide and wax wherever these solutions were in direct contact with oranges (Rajapakse, 2016). Despite these conclusions, the contribution of waxing steps to microbial food safety risks in fresh citrus remains poorly characterized.

Both storage and finishing waxes are used by the citrus industry (Cohen, 1988; Smilanick & Sorenson, 2001). Some citrus fruits (e.g., lemons) may be waxed twice. Diluted storage waxes can be applied to lemons without drying prior to as much as 6 months of storage under cool and humid conditions (Arpaia & Kader, 1999). Undiluted finishing waxes are applied to both lemons and oranges before final packing. The fruits are sorted and washed, and wax is applied by spraying the fruit and is then dried at 32–60°C for 2–3 min in a drying tunnel with forced air (personal communication with packinghouse personnel).

Survival of *Listeria monocytogenes* and *Salmonella* in diluted storage waxes or in full strength (undiluted) storage or finishing waxes depended on wax formulation and storage temperature, with greater

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survival observed at 4 than at 22°C in most cases (Sheng et al., 2023, 2024). *L. monocytogenes* survived better than *Salmonella* over 24 h at these temperatures in all but one of the 15 finishing waxes that were evaluated (Sheng et al., 2024).

Survival of microorganisms on citrus fruits during or after waxing is influenced by microorganism, wax type, and application method. Populations of *L. monocytogenes* (inoculated at ~6 log CFU/lemon) declined by 0.64–1.62 log on lemon surfaces right after waxing with four different diluted storage waxes (Sheng et al., 2023). Populations further declined to <1.30 log CFU/lemon after 28 or 75 days of storage at 12°C and ≥93% relative humidity (RH). In a survey of seven commercial orange or tangerine packinghouses in Florida, the application and drying of finishing wax reduced total aerobic plate counts and coliform counts from 3.7 log CFU/cm^2 and 35.2 most probable number (MPN)/cm^2, respectively, to 2.6 log CFU/cm^2 and 1.4 MPN/cm^2, respectively (Pao & Brown, 1998). An approximately 1-log reduction of inoculated *Escherichia coli* ATCC 25992 was observed after waxing oranges with a shellac-based water wax followed by drying with heated air at 52°C for 2 min.

Reductions of 0.4–5.9 log of inoculated *E. coli* ATCC 25992 were observed at the midsection and stem scar areas of Hamlin oranges, after dipping the fruit into an alkali (pH 8, 10, or 11) heated (25, 50, or 60°C) shellac-wax solution for 2 or 4 min (Pao et al., 1999). Greater reductions were observed at the midsection surface area and at higher pH, higher temperatures, and longer times. The authors did not compare the survival of *E. coli* ATCC 25992 to the survival of other microorganisms.

No significant difference was observed in the survival of a five-strain cocktail of *Salmonella* compared with a cocktail of *E. coli* strains ATCC 25922 and ATCC 35218 on the surface of grapefruit held at 20°C for 14 days (Danyluk et al., 2019). Similar reductions in both cocktails were also observed when grapefruit were treated with a range of wetting and washing steps under laboratory conditions; comparisons of the two organisms were not determined during the waxing of the fruit. Application of shellac-wax, or carnauba-wax with or without morpholine, to grapefruit along with a heated drying step in two different pilot-scale packing lines resulted in reductions of approximately 3.5 to >5 log of the inoculated *E. coli* cocktail (Danyluk et al., 2019).

Cross-contamination during waxing has been demonstrated in laboratory studies on noncitrus fruits. *Salmonella* transferred from inoculated cucumbers to brushes during cucumber waxing, irrespective of the wax type (mineral oil, vegetable oil, or petroleum wax) (Jung & Schaffner, 2021). A single apple inoculated with *L. monocytogenes* at 6.2 log CFU/apple transferred 3.6 log CFU/brush to a waxing brush, which then transferred 2.7–3.3 log CFU/apple to five sequential uninoculated apples (Shen et al., 2023).

The objectives of the present study were to evaluate, under laboratory conditions, the survival of *L. monocytogenes* or *Salmonella* on lemons or oranges after the application of different finishing waxes, after a heated drying step, and during subsequent fruit storage. To facilitate packinghouse trials, the survival of the surrogate organism *Enterococcus faecium* NRRL B-2354 was compared to the survival of the *Salmonella* during wax application and drying. The survival of *E. faecium* and the potential for cross- contamination between inoculated and uninoculated lemons during waxing and drying was then evaluated under pilot-scale packinghouse conditions.

Materials and methods

Citrus fruits and waxes

Unwashed and unwaxed lemons (Eureka) and oranges (Valencia) were obtained immediately after harvest from orchards at the University of California Lindcove Research and Extension Center (Exeter, CA). Upon receipt, the citrus fruits were stored at 4°C for less than 3 days prior to inoculation. The microbial populations of the citrus fruits were determined, as described below.

Four waxes (labeled as F4, F6, F8, and F15; Table 1) were chosen for this study to represent citrus or fruit waxes with different survival rates for *L. monocytogenes* and *Salmonella*, as previously determined in an evaluation of 15 undiluted fruit and citrus waxes during 24 h of storage at 4 and 22°C (Sheng et al., 2024). As recommended by the wax suppliers, all waxes were held in sealed containers at ambient temperature (22.0 ± 0.6°C) before use at full strength (undiluted). Upon receipt, the pH of each wax was measured with a pH meter (Mettler Toledo).

Bacterial cultures

Rifampin-resistant strains of *L. monocytogenes* and *Salmonella enterica* subsp. *enterica* (*Salmonella*) were used in the study (Supplemental Table S1). The parent strains were associated with fresh produce or tree nut outbreaks or recalls: *L. monocytogenes* (diced onion recall, whole cantaloupe, celery processing, and cantaloupe processing facilities associated with listeriosis outbreaks), *Salmonella* Enteritidis (almond outbreak), *Salmonella* Gaminara (orange juice outbreak), *Salmonella* Montevideo (tomato outbreak), *Salmonella* Muenchen (orange juice outbreak), and *Salmonella* Rubislaw (orange juice outbreak). In addition, a rifampin-resistant variant of the nonpathogenic surrogate strain *E. faecium* (NRRL B-2354) was used in the laboratory and pilot-plant trials. All cultures were stored at −80°C in tryptic soy broth (TSB) supplemented with 15% glycerol. Unless otherwise specified, culture media were Difco brand (BD).

Preparation of inocula

Individual frozen stock cultures were streaked onto tryptic soy agar supplemented with rifampin (Biosynth International) at 75 μg/mL (TSAR), and then incubated at 37°C overnight. An isolated colony from each culture was then transferred into 10 mL of TSB supplemented with rifampin at 75 μg/mL (TSBR) and incubated at 37°C for 24 h. One 10-μL loopful of the overnight culture was transferred to 10 mL of fresh TSBR and incubated at 37°C for another 24 h. After that, the overnight culture was spread onto four TSA plates (250 μL/plate), dried in the biosafety cabinet for 30 min, and then incubated at 37°C for 24 h. The resulting bacterial lawn was collected after adding 5 mL of 1 × phosphate-buffered saline (PBS, pH 7.4) to each plate and scraping the lawn with an L-shaped disposable spreader (VWR). The cells suspended in PBS were pipetted into a 15-mL Falcon tube (BD); average counts in each cell suspension after bacterial lawn collection in PBS are presented in Supplemental Table S2. To prepare the six-strain *L. monocytogenes* or five-strain *Salmonella* cocktail inoculum, equal volumes (1 mL) of each strain were combined. Serial dilutions of each inoculum were made in PBS to achieve target populations (ca. 8 log CFU/mL).

Citrus fruit inoculation

Lemons and oranges were removed from refrigerated storage and held at ambient temperature for 1 day prior to inoculation. One circle (2.0 cm in diameter) was drawn on the midsection surface of each lemon and orange to define the inoculation area, as previously described (Sheng et al., 2023). The prepared *L. monocytogenes*, *Salmonella*, or *E. faecium* inoculum was spot inoculated (10 1-μL drops) within each marked area to achieve an initial target level of ca. 6 log CFU/fruit. Inoculated lemons and oranges were placed in a shallow container covered loosely with a lid and held on a lab bench overnight (ca. 18 h) at ambient conditions (18.5 ± 1.8°C; 52–83% relative humidity [RH], median 80% RH), at which point no visible liquid was observed in the inoculated areas.

Table 1
pH range and wax information of finishing waxes

Finishing wax (source)[a]	pH	Log reduction of pathogens in finishing wax at 4°C during 24 h of storage [b]		Wax information
		L. monocytogenes	*Salmonella*	
F4 (A)	9.1–9.7	>6	>6	Resin-based, nonmorpholine gloss – proprietary ingredients
F6 (A)	8.4–9.2	<1	~1	Organic, shellac-based, nonmorpholine gloss – proprietary ingredients
F8 (A)	8.8–9.6	>6	~2.2	Organic, beeswax-based, nonmorpholine gloss – proprietary ingredients
F15 (B)	9.1–9.4	~2.5	>6	Carnauba-based, morpholine (<3%) – proprietary food-grade ingredients

[a] Waxes were provided by one of two commercial firms.
[b] Sheng et al., 2024.

In-lab waxing and drying – inoculated lemons

Lemons inoculated with *L. monocytogenes*, *Salmonella*, or *E. faecium* (ca. 6 log CFU/fruit) were waxed individually with one of the four selected finishing waxes. Briefly, each inoculated fruit was placed on its side (inoculated surface facing up) on a 118-mL (4-oz) sterile specimen container without the lid (Thermo Fisher Scientific). A finishing wax was then sprayed on the exposed half of the fruit for 10 s with an airbrush compressor (Central Pneumatic, Cheyenne, WY) held at ca. 10 cm above the surface. The fruit was flipped manually by 180 degrees and then sprayed with the finishing wax for 10 s, which delivered a total of ca. 0.8 mL of wax per fruit. Fruits that were inoculated but unwaxed, or inoculated and sprayed with water, were used as controls.

The inoculated and waxed or control lemons were dried by holding at 22°C for 7 min (nonheated control) or in a convection oven for dynamic heat drying at 50 or 60°C for 7 min (Fig. 1A). The oven temperature was monitored using three thermocouples (Omega Engineering Inc.) placed inside the oven chamber at three different locations. Populations of surviving *L. monocytogenes, Salmonella,* and *E. faecium* on the whole fruit were determined after inoculation, after waxing, and after drying.

In-lab waxing and storage of Salmonella–inoculated citrus fruits

To evaluate the survival of *Salmonella* on citrus fruit after in-lab finishing wax application, *Salmonella*-inoculated lemons or oranges (6 log CFU/fruit) were waxed with F6 using the same spray protocol as described above. Fruits that were inoculated but unwaxed, or inoculated and sprayed with water, were used as controls. The inoculated and waxed or control fruits were dried at 60°C for 7 min, as described above, and then stored for up to 30 days under refrigeration or at ambient conditions (Fig. 1B). Randomly selected lemons or oranges were sampled and populations of *Salmonella* were determined immediately after inoculation (before drying the inoculum; day −1), after drying the inoculum (day 0 before waxing and drying), after waxing and drying (day 0), and during storage (days 1, 3, 5, 10, 20, and 30). The temperature and RH during storage were recorded using data loggers (TempTale 4, Sensitech Inc.).

Pilot-scale waxing and drying trials – E. faeciuminoculated lemons

Three pilot-scale trials were conducted at the Lindcove Research and Extension Center during the 2022 lemon harvest season. Two days before the start of each pilot-scale trial, lemons were harvested at the Lindcove Research and Extension Center and then held at ambient conditions in the pilot plant. One day before each pilot trial, *E. faecium* was spot inoculated onto lemons at 6 log CFU/fruit, as described above, and the inoculated lemons were then labeled with a marker (with an "X") in a separate spot from the inoculation circle and dried overnight in the pilot plant at ambient conditions (14.2–16.7°C; 57–68% RH). Each waxing and drying trial had three consecutive runs through the line: the first run was with 100 or 150 uninoculated lemons, the second run was with 20% *E. faecium*–inoculated lemons (20 or 30, respectively) and 80% uninoculated lemons (80 or 120, respectively), and the third run was with 100 or 150 uninoculated lemons, respectively (Fig. 1C). Lemons were sampled after waxing and drying, and *E. faecium* was enumerated or detected using standard plating and enrichment methods, respectively , as described below. After the first run and third run, 15–20 uninoculated lemons were sampled and enriched for *E. faecium.* For the second run, a subset of 15–20 uninoculated lemons and 15–20 *E. faecium*–inoculated lemons were randomly sampled to enumerate the surviving *E. faecium*; the uninoculated lemons were also enriched for *E. faecium*. For each pilot trial, two swab samples (EZ Reach Sponge with 10 mL D/E broth; World Bioproducts, Woodinville, WA) were taken from each of four food contact surfaces both before the trial began and after all the lemons had been waxed. The waxing brush surface, waxing exit area, dryer exit, and postdryer conveyer belts were each sampled by swabbing over an area of 100 cm^2 (10 cm × 10 cm).

Microbiological analysis

Each whole fruit was placed into a 1,627-mL (55-oz) Whirl-Pak bag and 20 mL of Dey-Engley (D/E) neutralizing broth was added to neutralize the pH of applied wax. Sample bags were shaken by hand for 30 s, rubbed for 1 min, and shaken again for 30 s (referred herein as the shake-rub-shake method).

To determine the background aerobic bacteria and yeast and mold counts of lemons and oranges, the broth was plated onto plate count agar (PCA) and dichloran Rose Bengal chloramphenicol agar (DRBC) with a spiral plater (Interscience). PCA plates were incubated at 37°C for 48 h, and DRBC plates were incubated at ambient conditions (22 ± 0.6°C; 28–63% RH, median 54% RH) for 3–5 days. To determine the recovery of inoculated *L. monocytogenes*, *Salmonella*, and *E. faecium* on the whole lemons, or inoculated *Salmonella* on the whole oranges, the broth was spiral plated onto TSAR, as well as xylose lactose tergitol-4 (XLT4) agar supplemented with rifampin at 75 μg/mL for *Salmonella* or modified Oxford medium (MOX) supplemented with rifampin at 75 μg/mL for *L. monocytogenes*, and incubated at 37°C for 24 h. Colonies were counted, and bacterial populations were determined. When colony counts for TSAR were expected to be near or below the limit of detection (LOD) by plating (1.3 log CFU/fruit), 5 mL of the D/E was combined with 5 mL of double-strength enrichment broth supplemented with rifampin at 75 μg/mL (i.e., buffered *Listeria* enrichment broth for *L. monocytogenes*, lactose broth for *Salmonella*, and TSB for *E. faecium*) and then incubated at 37°C for 24 h, followed by streaking onto the selective agar or TSAR to determine the presence of target microorganisms (LOD of enrichment: 0.6 log CFU/fruit). For the swab samples, 225 mL of TSB supplemented with rifampin (75 μg/mL) was added to each swab sample and then

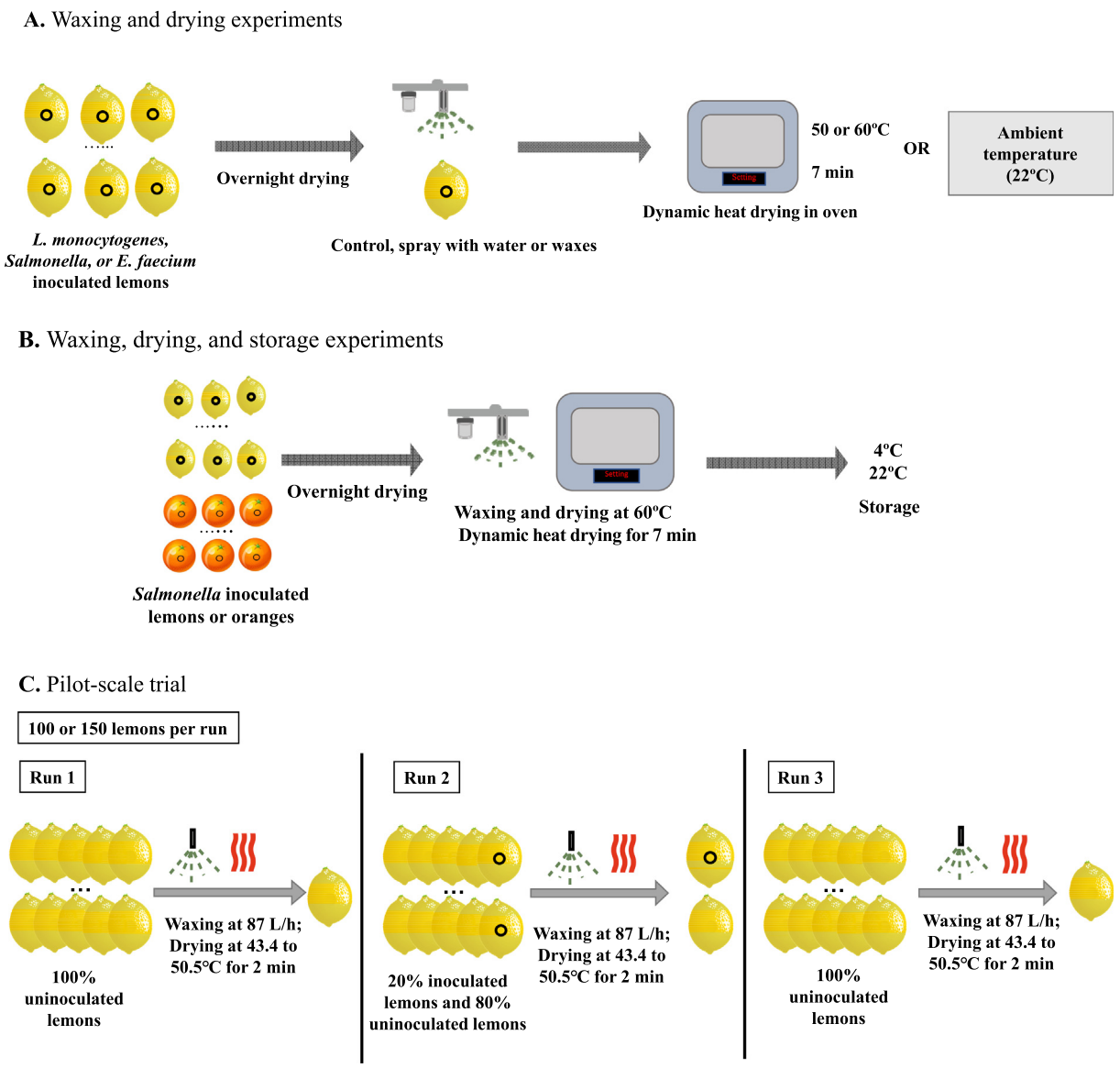

Figure 1. Schematic of experimental design for laboratory and pilot-scale packinghouse studies.

incubated at 37°C for 24 h, followed by streaking onto TSAR to determine the presence of target microorganisms.

Statistical analysis

Each laboratory experiment was performed in triplicate and repeated independently twice with different inoculum preparations ($n = 6$). Plate count data were converted to log numbers of CFU/fruit. The limit of detection by plating (LOD; 20 CFU/fruit or 1.3 log CFU/fruit) was determined assuming a single colony on quadruplicates of 250 µl per plate of the lowest dilution (20 mL/fruit). Log reductions were calculated by subtracting the $\log_{10}$ counts from individual test fruit from the mean of the $\log_{10}$ counts from the control fruit. The pilot-scale study consisted of three trials performed on three separate visits. In each trial, a total of three runs were conducted with 100 or 150 lemons for each run. After each run, 15–20 inoculated or uninoculated lemons were sampled ($n = 45$ or 60). The statistical analysis was performed with GraphPad Prism software 8.0. Comparisons of population levels were analyzed using Tukey-Kramer tests with JMP 14 Pro software (SAS Institute Inc.). Differences in the mean values were considered significant at $P < 0.05$.

Results

Background microbiota of citrus fruits

Populations of background total aerobic bacteria were 4.81 ± 0.82 log CFU/lemon and 4.92 ± 0.52 log CFU/orange. The yeast and mold counts were 4.59 ± 0.60 log CFU/lemon and 5.20 ± 0.51 log CFU/orange. No colonies were observed on MOX, XLT4, or TSAR at the lowest dilution (LOD = 1.30 log CFU/fruit).

Reduction of pathogens on lemons after in-lab waxing and drying

A heated drying step (32–60°C; 2–3 min) typically follows the application of finishing waxes to citrus fruits in commercial packinghouses (personal communication with packinghouse personnel). When the heat treatment was performed in a lab oven, it took approximately 3 min for the oven to return to the target temperature after the oven door was closed. Therefore, the 7-min dynamic heat drying step consisted of 3 min of come-up time and 4 min of heated drying at the predesignated temperature. Holding at 22°C for 7 min served as a nonheated control. Bacterial counts recovered from MOX or XLT4

were not significantly different than counts enumerated on TSAR except for four cases (~4%) where counts were significantly lower by ~0.5 log. Therefore, unless otherwise indicated, the data for TSAR are presented in the figures and in the text.

L. monocytogenes and *Salmonella* populations were 6.40 ± 0.04 and 6.03 ± 0.10 log CFU/lemon, respectively, after inoculation and before overnight drying (day −1). *L. monocytogenes* and *Salmonella* populations were recovered at 5.82 ± 0.25 and 5.68 ± 0.11 log CFU/lemon, respectively, from control lemons after overnight drying, before waxing and drying. The application of finishing wax, followed by subsequent holding (22°C) or drying (60°C) for 7 min, resulted in significantly ($P < 0.05$) higher reductions of both *L. monocytogenes* and *Salmonella* populations on waxed lemons compared with unwaxed or water-sprayed lemons (Fig. 2; Supplemental data). The magnitude of the reductions depended on the wax type and the treatment temperature. *L. monocytogenes* and *Salmonella* populations decreased by 0.94–1.77 and 0.27–1.27 log, respectively, on lemon surfaces immediately after waxing and without a holding or drying step. *L. monocytogenes* and *Salmonella* populations decreased by 1.99–3.09 and 0.54–2.63 log after waxing and holding at 22°C for 7 min, respectively. *L. monocytogenes* and *Salmonella* populations declined by 2.84–4.44 and 1.65–3.67 log after both waxing and drying at 60°C for 7 min, respectively. Waxing with F4 or F8 resulted in greater reductions of *L. monocytogenes* and *Salmonella* on lemon surfaces after holding or heated drying compared to waxing with F6 or F15 or to lemons in the control group. Significantly ($P < 0.05$) greater reductions were observed for *L. monocytogenes* than for *Salmonella* under most of the tested conditions. Therefore, *Salmonella* was selected for all subsequent lab and storage studies. Finishing wax F6 was selected for the subsequent storage studies to mimic the worst-case scenario because the smallest *Salmonella* reductions (1.56–1.65 log/lemon) were observed after waxing and drying when using this wax.

Reductions of Salmonella and E. faecium on lemons after in-lab waxing and drying

The application of finishing waxes F4, F6, and F8 without drying resulted in reductions of up to 1.46 log for *E. faecium* and 1.27 log for *Salmonella* (Fig. 3A; Supplemental data). Application of waxes F4 or F8, but not wax F6, led to significantly ($P < 0.05$) greater reductions of *E. faecium* compared to water-sprayed lemons. No significant differences were observed in reductions of *Salmonella* on lemons sprayed with any of the three waxes or with water.

After holding at 22°C or heated drying at 50 or 60°C for 7 min, significant reductions ($P < 0.05$) of both *Salmonella* and *E. faecium* were observed on lemons coated with the different finishing waxes compared with the unwaxed or water-sprayed control lemons. Reductions of *E. faecium* were 1.85, 0.86, and 2.27 log on F4-, F6-, and F8-waxed

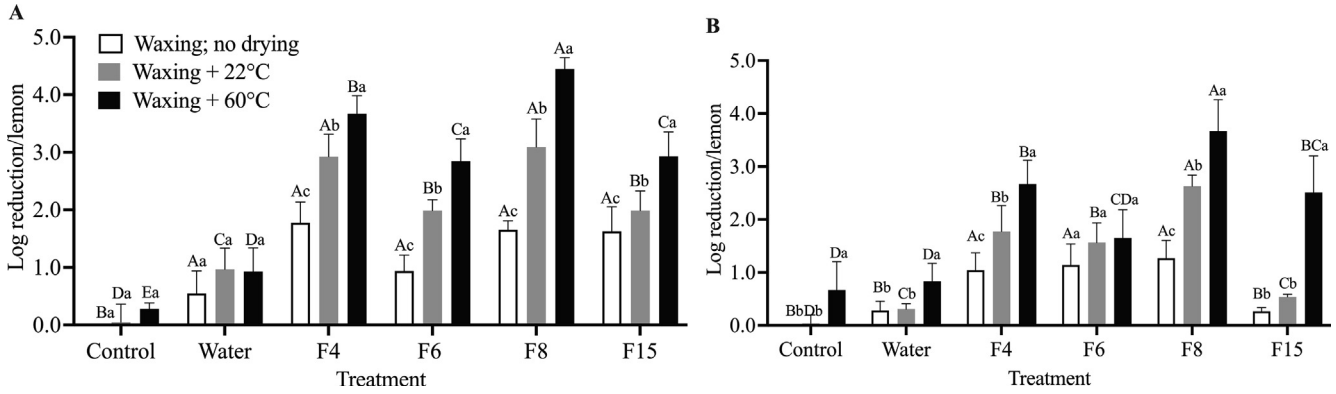

Figure 2. Reductions of *L. monocytogenes* (A) and *Salmonella* (B) on lemon surfaces after application of finishing wax (F4, F6, F8, or F15) combined with holding at 22°C for 7 min or drying at 60°C for 7 min; control lemons were unwaxed or sprayed with water ($n = 6$). Log reductions were calculated by subtracting the $\log_{10}$ counts from individual test lemons from the mean of the $\log_{10}$ counts from the unwaxed control lemons (after overnight drying). Within each postwaxing holding or drying temperature for each pathogen, log reductions with different uppercase letters are significantly different among wax types ($P < 0.05$). Within each treatment (control, water, or individual wax) for each pathogen, log reductions with different lowercase letters are significantly different among treatment temperatures ($P < 0.05$).

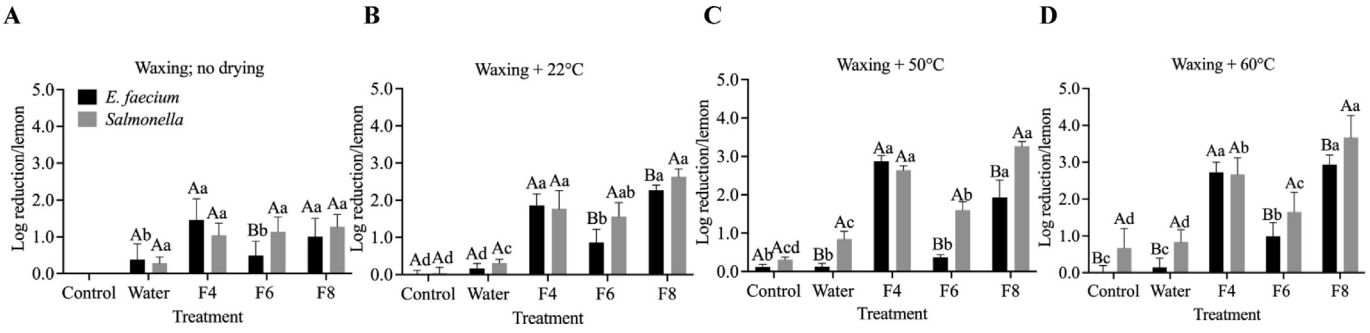

Figure 3. Reductions of *E. faecium* and *Salmonella* on lemon surfaces after application of finishing wax (F4, F6, or F8) without drying (A) or combined withholding at 22°C (B) or drying at 50°C (C) or 60°C (D). Control lemons were unwaxed or sprayed with water ($n = 6$). Log reductions were calculated by subtracting the $\log_{10}$ counts from individual test lemons from the mean of the $\log_{10}$ counts from the unwaxed control lemon (A). Within each treatment, log reductions with different uppercase letters are significantly different between microorganisms ($P < 0.05$). For each microorganism and within each waxing-drying treatment (A, B, C, D), log reductions with different lowercase letters are significantly different among treatments (control, water, or wax) ($P < 0.05$).

lemons, respectively, and reductions of *Salmonella* were 1.77, 1.56, and 2.63 log on F4-, F6-, and F8-waxed lemons, respectively, when held at 22°C (Fig. 3B). *E. faecium* populations declined by 0.37–2.87 log or 0.99–2.93 log when dried at 50 or 60°C, respectively (Fig. 3C). *Salmonella* populations declined by 1.60–3.27 log or 1.65–3.67 log, when dried at 50 or 60°C, respectively (Fig. 3D). Overall, greater reductions were observed for *Salmonella* than for *E. faecium*. Significantly greater reductions (data not shown; $P < 0.05$) of *E. faecium* were observed after drying at 60°C compared to 50°C when lemons were waxed with F8. No significant difference in reductions between 50 and 60°C was observed for *Salmonella*-inoculated lemons regardless of the wax type.

Survival of Salmonella on citrus after waxing, heated drying, and during subsequent storage

Salmonella populations were 5.93 ± 0.33 or 6.48 ± 0.08 log CFU/fruit on lemons (Table 2) or oranges (Table 3), respectively, after inoculation and before overnight drying (day −1). After overnight drying, *Salmonella* populations were 5.78 ± 0.57 or 5.93 ± 0.25 log CFU/fruit on control lemons or oranges, respectively (day 0). After drying at 60°C for 7 min, on day 0 of storage, *Salmonella* populations were 5.34 ± 0.25 or 5.58 ± 0.54 log CFU/fruit on control lemons or oranges, respectively. After waxing with F6 and drying at 60°C for 7 min, the populations of *Salmonella* on waxed lemons or oranges were 4.10 ± 0.30 or 4.23 ± 0.45 log CFU/fruit, respectively, representing reductions of 1.68 or 1.70 log for lemons or oranges, respectively, compared to the unwaxed control (day 0 after overnight drying).

The measured temperature and RH were 4.0 ± 0.2°C and 77–93% RH (median 78% RH) under refrigerated storage, and 21.0 ± 0.6°C and 30–99% RH (median 90% RH) under ambient storage. During 30 days of storage, *Salmonella* populations gradually decreased on both waxed and unwaxed lemons and oranges. Significantly greater reductions ($P < 0.05$) of *Salmonella* were observed on waxed lemon and orange surfaces when compared to unwaxed fruit surfaces, regardless of the fruit type or storage temperature (Tables 2 and 3). After 30 days of storage at 4°C, the reductions of *Salmonella* on waxed lemons or oranges were greater by 1.40–1.43 or 0.56–1.02 log, respectively, than reductions on the unwaxed citrus fruit (Tables 2, 3, and 4). The reductions of *Salmonella* on waxed lemons or oranges held at 22°C were greater by 0.18–0.29 or 0.54–0.57 log, respectively, than reductions on the unwaxed citrus fruits. The reduction of *Salmonella* populations was greater on both waxed lemons and oranges when the storage temperature was 4°C (Table 4). *Salmonella* populations decreased by >3.5 log after 30 or 20 days of storage at 4°C on waxed lemons (Table 2), or waxed oranges (Table 3), respectively. Populations of *Salmonella* decreased by 2.18 log on waxed lemons or by 2.80 log on waxed oranges after 30 days of storage at 22°C (Table 4). Significantly greater reductions ($P < 0.05$) of *Salmonella* were observed on waxed or control oranges than on waxed or control lemons during storage at both 4 and 22°C (Table 4).

Reductions of E. faecium on lemons after waxing and drying in pilot-scale trials

Microbial reductions are often higher under laboratory conditions than under pilot or commercial-scale scenarios. For this reason, waxes achieving higher reductions under laboratory conditions were considered for the pilot study to represent a best-case scenario. Significantly greater ($P < 0.05$) reductions of *E. faecium* under laboratory conditions were observed with waxes F4 and F8 compared with F6 (2.72 to 2.87 log/lemon for F4 and 1.93 to 2.93 log CFU/lemon for F8 with 50 or 60°C heated drying) (Fig. 3). Wax F4 and F8 are used in conventional and organic operations, respectively. Wax F4 was selected for the pilot-scale trials as there was no detectable background microflora in the wax by plating (LOD: 1.00 log CFU/mL;) whereas wax F8 had an aerobic plate count of 6 log CFU/mL (Sheng et al., 2024).

The temperature and RH conditions of the pilot plant during the trials were 14.0–16.7°C and 57.3%–68.6% RH, respectively (Table 5). The initial populations of *E. faecium* on lemons after inoculation and before drying were ~6 log CFU/lemon in each of the three trials (Table 5). *E. faecium* populations decreased by 0.45–0.63 log after

Table 2
Survival of *Salmonella* on inoculated lemons (log CFU/fruit) after treatment (i.e., control, water, or wax F6) and drying at 60°C for 7 min, followed by storage at 4 or 22°C for up to 30 days[a,b]

Temp (°C)	Storage time (day)	Control (untreated)		Water		Wax F6	
		TSAR [c]	XLT4 [c]	TSAR	XLT4	TSAR	XLT4
22	−1 (after inoculation)	5.93 ± 0.33	5.61 ± 0.24				
	0 [d]	^{A} 5.78 ± 0.57 ^{Aa}	^{A} 5.62 ± 0.33 ^{Aa}	^{A} 5.50 ± 0.3 ^{Aa}	^{A} 5.35 ± 0.18 ^{Aa}	^{A} 4.63 ± 0.46 ^{Ba}	^{A} 4.55 ± 0.52 ^{Ba}
	0 (after drying)	^{AB} 5.34 ± 0.25 ^{Aa}	^{A} 5.17 ± 0.25 ^{Aa}	^{AB} 5.28 ± 0.08 ^{Aa}	^{AB} 5.07 ± 0.18 ^{Aa}	^{A} 4.10 ± 0.30 ^{Ba}	^{B} 3.70 ± 0.37 ^{Ba}
4	1	^{BC} 5.01 ± 0.15 ^{Aa}	^{B} 4.49 ± 0.10 ^{Ab}	^{BC} 4.81 ± 0.58 ^{Aa}	^{BC} 4.49 ± 0.58 ^{Aa}	^{B} 2.98 ± 0.56 ^{Ba}	^{C} 2.88 ± 0.47 ^{Ba}
	3	^{BC} 4.91 ± 0.25 ^{Aa}	^{B} 4.57 ± 0.41 ^{Aa}	^{C} 4.25 ± 0.46 ^{Ba}	^{CD} 3.79 ± 0.61 ^{Ba}	^{C} 1.63 ± 0.53 ^{Ca}	^{D} 1.73 ± 0.47 ^{Ca}
	5	^{CD} 4.41 ± 0.51 ^{Aa}	^{B} 4.23 ± 0.54 ^{Aa}	^{C} 4.22 ± 0.53 ^{Aa}	^{C} 4.23 ± 0.54 ^{Aa}	^{C} 1.61 ± 0.36 ^{Ba}	^{D} 1.48 ± 0.28 ^{Ba}
	10	^{C} 4.59 ± 0.31 ^{Aa}	^{B} 4.55 ± 0.15 ^{Aa}	^{C} 4.15 ± 0.18 ^{Aa}	^{C} 3.97 ± 0.23 ^{Ba}	^{C} 1.85 ± 0.54 ^{Ba}	<1.30 [e]
	20	^{DE} 3.82 ± 0.41 ^{Aa}	^{C} 3.61 ± 0.27 ^{Aa}	^{D} 3.38 ± 0.26 ^{Aa}	^{DE} 3.22 ± 0.23 ^{Ba}	^{C} 1.50 ± 0.36 ^{Ba}	^{D} 1.38 ± 0.19 ^{Ca}
	30	^{E} 3.35 ± 0.12 ^{Aa}	^{D} 3.00 ± 0.28 ^{Aa}	^{D} 3.17 ± 0.21 ^{Aa}	^{E} 2.95 ± 0.35 ^{Aa}	<0.60 [f]	<0.60
22	1	^{AB} 5.44 ± 0.26 ^{Aa}	^{A} 5.14 ± 0.19 ^{Ab}	^{BC} 4.85 ± 0.54 ^{Aa}	^{BC} 4.58 ± 0.65 ^{Aa}	^{B} 3.30 ± 0.66 ^{Ba}	^{BC} 3.10 ± 0.54 ^{Ba}
	3	^{BC} 4.83 ± 0.26 ^{Aa}	^{CD} 4.20 ± 0.37 ^{Ab}	^{C} 4.33 ± 0.31 ^{Ba}	^{C} 4.34 ± 0.40 ^{Aa}	^{B} 3.04 ± 0.35 ^{Ca}	^{CD} 2.72 ± 0.25 ^{Ba}
	5	^{CD} 4.48 ± 0.36 ^{Aa}	^{BC} 4.53 ± 0.55 ^{Aa}	^{C} 4.52 ± 0.33 ^{Aa}	^{CD} 3.98 ± 0.23 ^{Ab}	^{BC} 2.63 ± 0.39 ^{Ba}	^{DE} 2.41 ± 0.30 ^{Ba}
	10	^{D} 4.10 ± 0.31 ^{Aa}	^{DE} 3.73 ± 0.55 ^{Aa}	^{D} 3.38 ± 0.12 ^{Ba}	^{E} 3.10 ± 0.15 ^{Ba}	^{C} 2.31 ± 0.17 ^{Ca}	^{DE} 2.18 ± 0.25 ^{Ca}
	20	^{DE} 3.99 ± 0.26 ^{Aa}	^{DE} 3.70 ± 0.25 ^{Aa}	^{D} 3.64 ± 0.31 ^{Aa}	^{DE} 3.40 ± 0.21 ^{Aa}	^{C} 2.05 ± 0.31 ^{Ba}	^{E} 1.85 ± 0.32 ^{Ba}
	30	^{E} 3.45 ± 0.28 ^{Aa}	^{E} 3.41 ± 0.38 ^{Aa}	^{D} 3.27 ± 0.10 ^{Aa}	^{E} 3.07 ± 0.30 ^{Aa}	^{C} 1.93 ± 0.22 ^{Ba}	^{E} 1.72 ± 0.32 ^{Ba}

[a] Lemons were stored at 4.1 ± 0.2°C, 82.4% ± 5.4% RH or 22.1 ± 0.1°C, 90.6% ± 2.3% RH.

[b] Values are means ± standard deviation ($n = 6$). Within columns for the same media and temperature, means with different uppercase letters before the values are significantly different over storage time at each temperature ($P < 0.05$). Within rows and media (TSAR or XLT4), means with different uppercase letters after the values are significantly different among different treatments ($P < 0.05$). Within treatments and sampling day, means with different lowercase letters after the values are significantly different between the media ($P < 0.05$).

[c] TSAR, tryptic soy agar supplemented with rifampin at 75 μg/mL; XLT4, xylose lysine tergitol-4 agar supplemented with rifampin at 75 μg/mL.

[d] Control lemons were evaluated after overnight drying, and water-sprayed or waxed lemons were evaluated after overnight drying and waxing.

[e] <1.30: All samples below the limit of detection (1.3 log CFU/fruit) by plating were positive for *Salmonella* by enrichment (0.60 log CFU/fruit).

[f] <0.60: All samples below the limit of detection (0.6 log CFU/fruit) were negative for *Salmonella* by enrichment.

Table 3
Survival of *Salmonella* on inoculated oranges (log CFU/fruit) after treatment (i.e., control, water, or wax F6) and drying at 60°C for 7 min, followed by storage at 4 or 22°C for up to 30 days[a,b]

Temp (°C)	Storage time (day)	Control		Water		Wax F6	
		TSAR[c]	XLT4[c]	TSAR	XLT4	TSAR	XLT4
22	−1 (after inoculation)	6.48 ± 0.08	6.05 ± 0.11				
	0 [d]	^{A} 5.93 ± 0.25 ^{Aa}	^{A} 5.58 ± 0.35 ^{Aa}	^{A} 5.83 ± 0.22 ^{Aa}	^{A} 5.71 ± 0.18 ^{Aa}	^{A} 5.48 ± 0.14 ^{Ba}	^{A} 5.27 ± 0.36 ^{Aa}
	0 (after drying)	^{A} 5.58 ± 0.54 ^{Aa}	^{AB} 5.29 ± 0.39 ^{Aa}	^{A} 5.74 ± 0.28 ^{Aa}	^{A} 5.49 ± 0.38 ^{Aa}	^{B} 4.23 ± 0.45 ^{Ba}	^{C} 3.80 ± 0.50 ^{Ba}
4	1	^{A} 5.56 ± 0.30 ^{Aa}	^{ABC} 4.99 ± 0.46 ^{Ab}	^{B} 4.90 ± 0.45 ^{Aa}	^{B} 4.76 ± 0.36 ^{Aa}	^{C} 3.47 ± 0.63 ^{Ba}	^{C} 3.38 ± 0.54 ^{Ba}
	3	^{B} 4.49 ± 0.72 ^{Aa}	^{BC} 4.68 ± 0.41 ^{Aa}	^{CD} 3.71 ± 0.67 ^{Aa}	^{C} 3.58 ± 0.27 ^{Ba}	^{D} 2.05 ± 0.26 ^{Ba}	^{D} 1.96 ± 0.22 ^{Ca}
	5	^{B} 4.47 ± 0.49 ^{Aa}	^{CD} 4.21 ± 0.48 ^{Aa}	^{C} 4.12 ± 0.38 ^{Aa}	^{C} 4.00 ± 0.33 ^{Aa}	^{DE} 1.53 ± 0.20 ^{Ba}	^{EF} 1.43 ± 0.21 ^{Ba}
	10	^{BC} 3.73 ± 0.56 ^{Aa}	^{EF} 3.30 ± 0.73 ^{Aa}	^{DE} 3.01 ± 0.52 ^{Ba}	^{D} 2.79 ± 0.53 ^{Aa}	<1.30 [e]	<1.30
	20	^{BC} 3.69 ± 0.15 ^{Aa}	^{DE} 3.49 ± 0.23 ^{Aa}	^{E} 2.55 ± 0.23 ^{Ba}	^{D} 2.35 ± 0.10 ^{Ba}	<0.60 [f]	<0.60
	30	^{C} 2.96 ± 0.38 ^{Aa}	^{F} 2.66 ± 0.29 ^{Aa}	^{E} 2.68 ± 0.34 ^{Aa}	^{D} 2.24 ± 0.46 ^{Aa}	<0.60	<0.60
22	1	^{AB} 5.55 ± 0.18 ^{Aa}	^{AB} 5.09 ± 0.10 ^{Ab}	^{AB} 5.58 ± 0.49 ^{Aa}	^{AB} 5.44 ± 0.41 ^{Aa}	^{B} 4.67 ± 0.29 ^{Ba}	^{B} 4.53 ± 0.17 ^{Aa}
	3	^{BC} 5.19 ± 0.18 ^{Aa}	^{B} 4.63 ± 0.21 ^{Ab}	^{B} 5.16 ± 0.27 ^{Aa}	^{B} 4.84 ± 0.39 ^{Aa}	^{C} 3.42 ± 0.32 ^{Ba}	^{D} 3.03 ± 0.18 ^{Bb}
	5	^{C} 4.48 ± 0.36 ^{Aa}	^{B} 4.58 ± 0.49 ^{Aa}	^{C} 4.20 ± 0.21 ^{Aa}	^{C} 3.98 ± 0.23 ^{Ba}	^{D} 2.52 ± 0.24 ^{Ba}	^{EF} 2.18 ± 0.29 ^{Ca}
	10	^{D} 3.90 ± 0.43 ^{Aa}	^{C} 3.81 ± 0.27 ^{Aa}	^{CD} 4.09 ± 0.37 ^{Aa}	^{C} 3.85 ± 0.41 ^{Aa}	^{CD} 2.91 ± 0.39 ^{Ba}	^{DE} 2.74 ± 0.44 ^{Ba}
	20	^{D} 3.81 ± 0.30 ^{Aa}	^{CD} 3.57 ± 0.35 ^{Aa}	^{CD} 4.08 ± 0.29 ^{Aa}	^{C} 3.91 ± 0.36 ^{Aa}	^{E} 1.76 ± 0.32 ^{Ba}	^{F} 1.65 ± 0.35 ^{Ba}
	30	^{D} 3.31 ± 0.07 ^{Aa}	^{D} 2.96 ± 0.34 ^{Ab}	^{D} 3.52 ± 0.42 ^{Aa}	^{C} 3.14 ± 0.28 ^{Aa}	^{F} 1.43 ± 0.21 ^{Ba}	<1.30

[a] Oranges were stored at 4.0 ± 0.2°C, 80.4% ± 4.7% RH or 21.1 ± 0.5°C, 89.6% ± 3.9% RH.
[b] Values are means ± standard deviation ($n = 6$). Within columns for the same media and temperature, means with different uppercase letters before the values are significantly different over storage time at each temperature ($P < 0.05$). Within rows and media (TSAR or XLT4), means with different uppercase letters after the values are significantly different among different treatments ($P < 0.05$). Within treatments and sampling day, means with different lowercase letters after the values are significantly different between the media ($P < 0.05$).
[c] TSAR, tryptic soy agar supplemented with rifampin at 75 μg/mL; XLT4, xylose lysine tergitol-4 agar supplemented with rifampin at 75 μg/mL.
[d] Samples were taken after overnight drying for control oranges, and after overnight drying and waxing for water-sprayed or waxed oranges.
[e] <1.30: All samples below the limit of detection (1.3 log CFU/fruit) by plating were positive for *Salmonella* by enrichment (0.60 log CFU/fruit).
[f] <0.60: All samples below the limit of detection (0.6 log CFU/fruit) were negative for *Salmonella* by enrichment.

Table 4
Log reductions of *Salmonella* on inoculated lemons and oranges treated with wax F6 and stored for 30 days[a]

Step	Temp (°C)	Fruit	Control		Water		Wax F6	
			TSAR[b]	XLT4[b]	TSAR	XLT4	TSAR	XLT4
Application [c]	22	Lemons	NA[d]	NA	0.28 ± 0.30	0.27 ± 0.18	1.16 ± 0.46	1.07 ± 0.52
		Oranges	NA	NA	0.10 ± 0.22	0.03 ± 0.18	1.04 ± 0.27	0.84 ± 0.22
Drying [e]	60	Lemons	0.45 ± 0.25	0.45 ± 0.25	0.22 ± 0.08	0.28 ± 0.18	0.53 ± 0.30	0.85 ± 0.37
		Oranges	0.35 ± 0.54	0.30 ± 0.40	0.08 ± 0.28	0.16 ± 0.37	0.67 ± 0.44	0.94 ± 0.50
Storage [f]	4	Lemons	2.07 ± 0.17 ^{Ba}	2.18 ± 0.28 ^{Aba}	2.10 ± 0.21 ^{Ba}	2.13 ± 0.35 ^{Ba}	>3.50	>3.11
		Oranges	2.61 ± 0.38 ^{Aa}	2.63 ± 0.29 ^{Aa}	3.07 ± 0.34 ^{Aa}	3.25 ± 0.46 ^{Aa}	>3.63	>3.20
	22	Lemons	1.89 ± 0.27 ^{Ba}	1.77 ± 0.38 ^{Ba}	2.00 ± 0.10 ^{Ba}	2.00 ± 0.30 ^{Ba}	2.18 ± 0.22 ^{Ba}	1.98 ± 0.32 ^{Ba}
		Oranges	2.26 ± 0.07 ^{Aba}	2.32 ± 0.34 ^{Aa}	2.23 ± 0.42 ^{Ba}	2.35 ± 0.28 ^{Ba}	2.80 ± 0.20 ^{Aa}	2.50 ± 0.00 ^{Aa}

[a] Values are means ± standard deviation ($n = 6$).
[b] TSAR, tryptic soy agar supplemented with rifampin at 75 μg/mL; XLT4, xylose lysine tergitol-4 agar supplemented with rifampin at 75 μg/mL.
[c] Log reduction of *Salmonella* after application of water or wax: calculated by subtracting $\log_{10}$ counts from individual lemons or oranges after water spraying or waxing from the mean of the $\log_{10}$ counts from day 0.
[d] NA, not applicable.
[e] Log reduction of *Salmonella* after drying: calculated by subtracting $\log_{10}$ counts from individual lemons or oranges after drying from the mean of the $\log_{10}$ counts of water-sprayed or waxed lemons.
[f] Log reduction of *Salmonella* during storage: calculated by subtracting $\log_{10}$ counts from individual lemons or oranges after 30 days of storage from the mean of the $\log_{10}$ counts of lemons after waxing/water-spraying and drying at 60°C for 7 min. Within rows and media (TSAR or XLT4), for the reductions during storage, means with different uppercase letters are significantly different between lemons and oranges stored at 4 or 22°C ($P < 0.05$). Means with different lowercase letters are significantly different between the media ($P < 0.05$).

overnight drying (Table 5) to 5.5 to 5.6 log CFU/lemon; the decline was significant ($P < 0.05$) for Trial 2 but not Trials 1 or 3. No significant difference in *E. faecium* populations was observed after overnight drying (before pilot-scale waxing and drying) among the three trials.

Wax was applied at a rate of 87 L/h, with inlet and outlet temperatures at 43.4 and 50.5°C, respectively. Uniform distribution of wax on the lemons was visually observed after waxing and drying. Run 1 (uninoculated lemons) was used to determine if there were resident rifampin-resistant *E. faecium* in the waxing and drying lines. *E. faecium* could not be recovered by enrichment of 15 (Trial 1) or 20 (Trials 2 or 3) uninoculated lemons after Run 1 of each trial, confirming that the waxing equipment was not contaminated. Minimum reductions of *E. faecium* on inoculated lemon surfaces after the application of finishing wax (F4) combined with a drying step ranged from 1.64–2.12 log (Fig. 4). Mean reductions of *E. faecium* were significantly lower ($P < 0.05$) in Trial 1 than in Trials 2 and 3. *E. faecium* was recovered by whole-fruit enrichment but not by plating from the uninoculated lemons run with (Run 2) or after (Run 3) the inoculated lemons. *E. faecium* was recovered from 80% (44 of 55) and 84% (46 of 55) of the uninoculated lemons in Run 2 and Run 3, respectively. *E. faecium* NRRL B-2354 was not recovered from any of the eight swabs collected prior to the start of each of the three trials (24 total swabs; Supplemental Table S3). However, the organism was recovered from swabs collected after the trials from the waxing brush roller (5/6), waxing exit (5/6), dryer exit (3/6), and postdryer conveyor belt (6/6).

Table 5
Ambient conditions and *E. faecium* populations (log CFU/lemon[a]) on lemon surfaces after overnight drying in the pilot plant

Pilot trial	Pilot-plant temp and RH	After inoculation	After overnight drying
1	16.7 ± 1.5°C, 57.3% ± 4.4% RH	6.08 ± 0.31 ^{Aa}	5.56 ± 0.21 ^{Aa}
2	14.0 ± 2.0°C, 66.4% ± 3.5% RH	6.18 ± 0.12 ^{Aa}	5.55 ± 0.05 ^{Ba}
3	14.2 ± 2.3°C, 68.6% ± 7.0% RH	5.93 ± 0.39 ^{Aa}	5.48 ± 0.17 ^{Aa}

[a] Values are means ± standard deviation (n = 5); values represent enumeration on tryptic soy agar supplemented with rifampin at 75 μg/mL (TSAR). Within rows, means with different uppercase letters are significantly different between sampling time ($P < 0.05$). Within columns, means with different lowercase letters are significantly different between trials ($P < 0.05$).

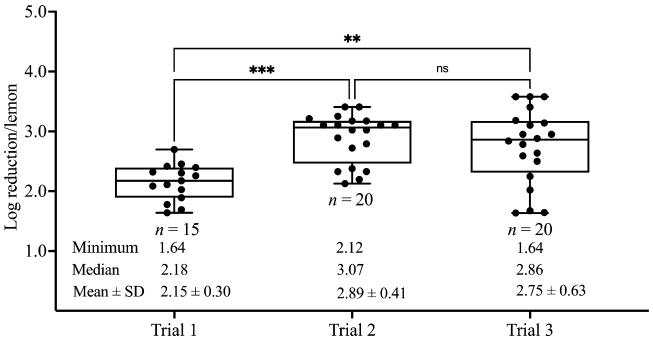

Figure 4. Reductions of *E. faecium* on inoculated lemon surfaces after the application of finishing wax (F4, 87 L/h) combined with a drying step at 43.3–50.5°C in pilot-scale trials. The mean initial level of *E. faecium* was 5.53 ± 0.04 log CFU/lemon across the three trials. For each trial, log reductions were calculated by subtracting the $\log_{10}$ counts from individual test lemons from the mean of the $\log_{10}$ counts from the unwaxed control lemon for that trial. For each boxplot, the maximum and minimum values are denoted by the whiskers; the 75th and 25th percentiles are denoted by the top and bottom of the box, respectively; and the median is denoted by the center line. The effect of different trials on reductions was tested by multiple comparison analysis, and the significance levels were added on the boxplot (ns, no significant difference with $P > 0.05$; ** significant difference with $P < 0.05$; *** significant difference with $P < 0.005$). The descriptive statistics (minimum, median, and mean ± SD) of log reductions are included below the figure.

Discussion

Wax application practices can differ among types of produce. The application of finishing waxes is usually followed by a heating step (32–60°C) in a tunnel that is often equipped with a fan to facilitate drying. Drying takes place over a few minutes (duration impacted by the length of the drying tunnel) to help set the wax. The uniform distribution and dryness of wax on citrus fruits are visually checked after completing the waxing and drying steps. In the present study, due to biosafety concerns, a conventional oven without a blower was used in the laboratory for the drying step. Thus, the drying time was expected to differ from the times commonly used in packinghouses. Dynamic heat drying at 50–60°C for 7 min resulted in visually uniformly dried wax on lemons in preliminary experiments (data not shown) and thus were used as the drying protocol.

The selection of finishing waxes in the present study was based on the observed log reduction of *L. monocytogenes* and *Salmonella* in finishing waxes held at 4°C for 24 h (Table 1; Sheng et al., 2024). Waxes that covered the range of outcomes were selected: F4 (>6-log reductions of both pathogens), F6 (~1-log reduction of both pathogens), F8 (significantly greater reduction of *L. monocytogenes* compared to *Salmonella*), and F15 (significantly greater reduction of *Salmonella* compared to *L. monocytogenes*). In general, the survival pattern for the two pathogens in the waxes did not correspond with the reductions observed on inoculated lemons. Significantly greater reductions on lemons were consistently observed for *L. monocytogenes* compared with *Salmonella*; generally, greater reductions of both *L. monocytogenes* and *Salmonella* were observed after the application of F4 and F8, with smaller reductions observed with the application of F6 and F15 after waxing and drying.

Bacterial species, holding or drying temperature, and wax type influenced the observed reduction of pathogens on lemon surfaces after waxing. Population reductions of 0.27–1.77 log were observed with the application of finishing waxes without holding or drying. In most cases, the application of finishing wax to lemons followed by drying at 50 or 60°C resulted in additional significant reductions of both *L. monocytogenes* (2.84–4.45 log) and *Salmonella* (1.6–3.67 log) compared with holding at 22°C. For this reason, *Salmonella* was selected as the pathogen of focus for storage studies following waxing and drying.

Similar observations were reported for waxed apples. The application of wax coating on *L. monocytogenes*-inoculated apples resulted in a 1.2-log reduction of *L. monocytogenes* (Macarisin et al., 2019). Drying at 55°C resulted in additional reductions of 1.0 and 0.5 log of *E. coli* O157:H7 and *Salmonella* Muenchen, respectively, on waxed apples compared to those held at 21°C (Kenney & Beuchat, 2002). In contrast, drying at 22, 45, or 60°C had no impact on the populations of *Listeria innocua* on waxed apples (Su et al., 2023). The differences in test microorganisms, fruit surfaces, wax types, and experimental design might explain the different observations in the reported studies.

Differences in susceptibility to the wax treatment among different microorganisms highlight the importance of studying more than one pathogen in food safety assessments, as distinct microorganisms may exhibit different behaviors to treatment conditions. In addition, selecting a suitable surrogate microorganism is crucial for evaluating the impact of unit operations on foodborne pathogens at a pilot or commercial scale. The ideal surrogate microorganism should have a long history of safe use and share similar characteristics with the tested pathogen under the target conditions (Hu & Gurtler, 2017). The surrogate microorganism should be genetically stable, easy to prepare to a suitable inoculum level, easy to enumerate, and have no adverse effects on the processing environment (Busta et al., 2003). *E. faecium* NRRL B-2354 does not possess genomic and phenotypic characteristics that have been linked to nosocomial *Enterococcus* infections (Kopit et al., 2014) and is considered a nonpathogen biosafety level 1 organism. To evaluate the reduction of *E. faecium* NRRL B-2354 with that of the target pathogen under the same treatment conditions, a laboratory study is usually needed (Sinclair et al., 2012). *E. faecium* NRRL B-2354 has been shown as an appropriate surrogate for *Salmonella* Typhimurium during postharvest interventions for fresh produce such as butternut squash or tomato washing (Stearns et al., 2023; Temple et al., 2023), and for *L. monocytogenes* in apple washing systems (Su et al., 2024).

In the lab-scale waxing and drying trials, heated drying at 50 or 60°C for 7 min resulted in substantial reductions of both *Salmonella* and *E. faecium* NRRL B-2354 on waxed lemons. Smaller reductions in *E. faecium* NRRL B-2354 compared with *Salmonella* were observed under the tested treatment conditions, suggesting that *E. faecium* NRRL B-2354 could serve as a conservative surrogate for *Salmonella* during finishing wax application. To the best of our knowledge, this was the first study to evaluate *E. faecium* NRRL B-2354 as a surrogate for *Salmonella* during finishing wax application and drying.

In the pilot-plant trials, the initial inoculation level of *E. faecium* NRRL B-2354 (5.5 log CFU/lemon) at the midsection was selected to represent a worst-case level, point source microbial contamination on lemons. A minimum of 1.6-log reduction of *E. faecium* NRRL B-

2354 (mean reductions from 2 to 3 log) was observed across the three pilot plant trials after application of the finishing wax F4 and drying. Similarly, the packinghouse application and heated drying of a finishing wax on oranges reduced total aerobic plate counts and coliform counts by approximately 1 log (Pao & Brown, 1998). In contrast, the application of shellac-, carnauba-, or morpholine with carnauba-based waxes and heated drying resulted in a ≥5-log reduction of inoculated *E. coli* on grapefruit (Danyluk et al., 2019). The differences among studies might be attributed to the different waxes, citrus fruit types, and microorganisms that were evaluated.

Care should be taken to extrapolate the pilotscale results to anticipated commercial outcomes. Wax F4 was selected for the pilot-scale study because greater reductions after waxing and drying under laboratory conditions were observed on lemons compared with wax F6. In addition, greater reductions of inoculated microorganisms were observed when the inoculum was applied at the midsection compared with application at the stem end of oranges after dipping the fruit into heated alkali (50°C; pH 10) wax solution for 4 min (Pao et al., 1999). Therefore, the reductions observed in the present study may differ from what could be expected at commercial facilities when other waxes are applied or when contamination occurs at the stem end of the fruit.

Although waxing may reduce microbial populations on fruit surfaces, this step can also be a potential point for cross-contamination. During pilot-scale waxing, *E. faecium* NRRL B-2354 was transferred from inoculated lemons to uninoculated lemons run with or after the inoculated lemons. Cross-contamination from inoculated fresh produce to the waxing brush and from the waxing brush to uninoculated fresh produce during subsequent waxing was also reported during cucumber or apple waxing (Jung & Schaffner, 2021; Shen et al., 2023).

After waxing the inoculated lemons, *E. faecium* NRRL B-2354 also was recovered from most (79%) of the swabs collected from food contact surfaces, including the waxing brush roller, waxing and dryer exit, and postdryer conveyor belt. Brush rollers are used with an overhead wax sprayer to uniformly distribute the wax. The wax polishing brushes in a packing line have been shown to trap the wax and *Listeria* cells (Ruiz-Llacsahuanga et al., 2021). Although not tested with wax, the transfer of *Salmonella* from inoculated tomatoes (~3 log CFU/g) to different roller conveyors (plastic, foam, or brush) was reported, and the transfer level was dependent on the conveyor material (Wang & Ryser, 2014).

After finishing wax application, fresh citrus is usually shipped to retail markets, often under refrigerated conditions (USDA, 2014). Once at retail, storage is usually at ambient temperature. Finishing wax F6 was selected to determine the impact of waxing on survival during storage because it resulted in the lowest reductions in *Salmonella* after application and drying (1.60–1.65 log per lemon). Oranges were included in the survival studies to evaluate the impact of fruit. Greater reductions of *Salmonella* were observed with waxed lemons and oranges than with unwaxed citrus fruits after 30 days of storage regardless of storage temperature and fruit type, with the greatest reductions for waxed lemons or oranges stored at 4°C and significantly faster declines on oranges than lemons. Overall reductions of >4 log were observed after waxing (F6), drying, and storage at 4°C for 30 days on both lemons and oranges; 2.0 to 2.3 log more than that observed for untreated or water controls. In a previous study, *L. monocytogenes* declined more rapidly in finishing wax F6 over 135 days of storage when the storage temperature was 22°C compared with 4°C (Sheng et al., 2024). The difference may be due to the difference in testing microorganisms, and the exposure of the organism to the wax, including both the volume and the drying step. However, the results indicate that outcomes observed in waxes may not translate to outcomes after application to fruit.

In other studies, the application of wax to fruits has resulted in increased or decreased survival of pathogens during storage, depending on the wax, fruit type, and storage temperature. *L. innocua* populations recovered from unwaxed apples were 0.2–0.5 log higher than those recovered from waxed apples after 18 weeks of commercial storage at 0.6°C and ~90% RH (Su et al., 2023). *L. monocytogenes* populations recovered from waxed Red Delicious, Granny Smith, and Fuji apples were 1–2 log higher than from the unwaxed apples after 160 days of storage at 3°C (Macarisin et al., 2019). Significantly greater populations of *Salmonella* Newport were recovered from waxed cucumbers than from unwaxed cucumbers after 24-h holding at room temperature (Callahan & Micallef, 2019). In contrast, *Salmonella* Muenchen populations declined by 0.9 and 0.5 log on unwaxed and waxed apples, respectively, after 6 weeks of storage at 21°C (Kenney & Beuchat, 2002). Populations of *Salmonella* on waxed and unwaxed cucumbers decreased by 3.7–4.5 and 2.9–3.3 log, respectively, after storage at 7 and 21°C for 7 days (Jung & Schaffner, 2021).

In the present study, the observed reduction of pathogens on lemon surfaces after finishing wax application (waxing and drying) was influenced by bacterial species, holding or drying temperature; reductions were generally *L. monocytogenes* > *Salmonella* > *E. faecium*. The results suggested that *E. faecium* NRRL B-2354 could be used as a conservative surrogate microorganism for *Salmonella* for finishing wax application. The observed bactericidal effect under laboratory conditions was demonstrated in pilot-scale studies. The pilot-scale application of one tested finishing wax coupled with a heated drying step significantly reduced the number of *E. faecium* NRRL B-2354 on lemon surfaces. However, cross-contamination between contaminated and uncontaminated lemons was observed during the waxing step. During storage for 30 days, greater reductions of *Salmonella* were observed on waxed lemon and orange surfaces when compared to unwaxed fruit surfaces, regardless of the fruit type and storage temperature. Populations fell below the limit of detection within 30 days of storage at 4°C on both fruits representing a >4-log reduction in inoculated and dried populations on the unwaxed controls. Although this observation was limited to a single tested wax (F6), other waxes included in the study (F4, F8, and F15) resulted in significantly greater reductions of microorganisms after waxing and drying (before storage).

Outcomes of the present study provide important information on the food safety role played by finishing wax application steps in packinghouses. The magnitude of the decline of *Salmonella* on citrus fruits during application and drying, and during subsequent fruit storage, depended on the finishing wax. These data suggest the waxing step may provide benefits in reducing pathogen contamination on citrus fruits. However, the waxing step also may provide an opportunity for cross-contamination. Both the selection of waxes and the waxing area in the packinghouse should be assessed in the facility food safety management program, and effective cleaning, sanitation, and environmental monitoring protocols applied.

CRediT authorship contribution statement

Hongye Wang: Writing – original draft, Visualization, Formal analysis, Data curation. **Lina Sheng:** Writing – review & editing, Data curation. **Zhuosheng Liu:** Data curation. **Xiran Li:** Data curation. **Linda J. Harris:** Writing – review & editing, Supervision, Funding acquisition. **Luxin Wang:** Writing – review & editing, Supervision, Funding acquisition.

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfp.2024.100255.

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