

Project Title:

Characterization and mitigation of food safety risks associated with waxing roller brushes

Project Period:

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Objectives:

1. Investigate the behavior of *Listeria monocytogenes* on waxing roller brushes made with different materials in the presence of fresh or built-up waxes.
2. Evaluate the levels of indigenous microbial populations present on commercial waxing roller brushes in commercial packinghouses.
3. Characterize the physical and chemical properties of hydrolyzed waxes, and optimize and validate the cleaning and sanitizing protocols for effective removal of waxes, wax residues, and microorganisms from waxing roller brushes.

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FINAL REPORT

Summary of Findings and Recommendations

- Both brush materials (50% horsehair/50% polyethylene [50/50] vs. nylon) and the presence of carnauba waxes impacted the behavior of *Listeria monocytogenes* (LM) on waxing roller brushes.
 - LM declined by approximately 2.5 log on unwaxed 50/50 brush quarters and 4.5 log on unwaxed nylon brush quarters by the end of 84 days of storage at ambient temperature.
 - LM declined by approximately 5 log on waxed 50/50 brushes and by 5.5 log on waxed nylon brushes over 84 days of storage at ambient temperature.
- Brush patterns (tufted vs. spiral) also impacted the bacterial behavior on waxing roller brushes. The artificially inoculated *Enterococcus faecium* (EF) declined by ~1.5 log CFU/brush coupon on spiral nylon brush coupons compared with the ~1.7 log reduction observed on tufted nylon brush coupons over 90 days of storage at ambient temperature.
 - The impact of brush patterns was also observed on 50/50 brushes. EF declined by ~1 and 0.5 log CFU, respectively, on spiral and tufted 50/50 brush coupons.
- Native microbial populations in packing facilities changed in response to cleaning and sanitation practices during harvest seasons. Although no significant difference was observed in culture-based plate counting results when samples were taken before and after regular cleaning and sanitation practices at some packing facilities, sequencing analysis showed that cleaning and sanitation practices significantly altered the microbiome composition.
- Intensive practices such as cleaning out of place (COP) and brush scrubbing significantly reduced the levels of culturable microorganisms.
- 50/50 brush filaments generally retained more carnauba-based, mineral-oil-based, and vegetable-oil-based wax than nylon brush filaments.
- When wax is wet (right after application), water rinsing removed >99% of the wet waxes regardless of the wax types (carnauba, mineral oil, or vegetable oil-based).
- When wax is dried (24 hours of drying at ambient temperature), carnauba-based wax was the most difficult one to remove.
- Alkaline cleaner was more effective for removing dried waxes compared to water and neutral cleaner.
- Soaking in alkaline cleaner with elevated temperatures (50 °C) or ultrasound could improve the removal efficacy of dried carnauba waxes.
- Pilot-plant validation study confirmed the efficacy of cleaning and sanitation protocols for waxing roller brushes when waxes were still wet.

Abstract

Waxing roller brushes can be difficult to clean and sanitize. Recent literature reported that *Listeria* spp. was isolated more frequently around the waxing area in packing facilities. To better characterize risks associated with waxing roller brushes, this study first investigated the impact of brush materials and brush patterns as well as the presence of waxes on the survival of *Listeria monocytogenes* (LM) or its surrogate *Enterococcus faecium* (EF). Results showed that LM survived better on 50% horsehair/50% polyethylene (50/50) brushes than on nylon brushes. The presence of dried carnauba waxes led to faster and greater reduction of LM during ambient storage. The impact of brush patterns on the attachment and survival of bacteria was conducted by using surrogate EF due to laboratory safety concerns. Results demonstrated that brush patterns (spiral vs. tufted) impacted the initial attachment and the survival of

EF during storage. Although interactions among brush patterns and brush materials were observed, EF generally had a greater reduction on spiral brushes than on tufted brushes, regardless of brush materials. Commercial packinghouse sampling was conducted to assess the levels of indigenous microbial populations on waxing roller brushes before and after cleaning and sanitation across the harvesting season. Intensive cleaning and sanitation practices, such as cleaning out of place or scrubbing of the brushes during cleaning, significantly reduced the culturable microbial levels. Sequencing analysis further indicated the changes in microbial compositions before and after cleaning and sanitation. Furthermore, in-lab and pilot-scale validation studies were carried out to evaluate the efficacy of cleaning and sanitation protocols. When waxes were wet, water rinsing alone could remove >99% of the waxes. When waxes were dried (24 hours of drying), alkaline cleaner was needed to remove dried carnauba waxes. Once the waxes were dried, 15-min sanitation with chlorine or peracetic acid had limited decontamination efficacy.

Background

In recent years, greater attention has been paid to post-harvest packinghouses, as several outbreaks and recalls were traced back to cross-contamination in packinghouses (Critzler et al., 2024). A recent study conducted by Ruiz-Llacsahuanga et al. (2021) at apple packinghouses found that *Listeria* spp. were most frequently isolated from the waxing area, compared with other areas such as washing, drying, and sorting. A recent CPS project (titled “Waxing of whole produce and its involvement in and impact on microbial food safety”) also found that pathogens such as *Listeria monocytogenes* (LM) and *Salmonella* could survive in waxes for extended periods of time. Most importantly, if the incoming fruits were contaminated, the waxing tunnel could serve as a cross-contamination spot among contaminated fruit, waxing roller brushes, and uncontaminated fruit (Wang et al., 2024).

Waxing roller brushes are usually made from synthetic polymers, natural fibers, or a combination of both. Nylon filaments offer the longest life and best bend recovery of all synthetic filaments (Brush Expert, 2025). Polyethylene brushes are generally softer yet durable, whereas natural horsehair brushes have the softest texture and are less tolerant of prolonged use. The blended material, 50% horsehair/50% polyethylene (50/50) mixture, is often described as highly absorbent and well-suited for wax application (Industrial Brush Corporation, 2025). During waxing, wax is sprayed from the sprinkler and spread evenly onto the brushes first, making sure all brushes are fully saturated with the wet waxes. After that, fruits are waxed by rolling and turning on the waxing roller brushes. Unfortunately, wax-saturated brushes pose challenges for the industry in cleaning and sanitation.

Waxes can be formulated from natural bases (e.g., carnauba, vegetable oil, shellac, or resin) or synthetic bases (e.g., petroleum-based waxes) (Irimia et al., 2025). These materials are mainly composed of long-chain hydrocarbon polymers with different functional groups. During cleaning, hydrolysis reaction (e.g., alkaline hydrolysis) can cleave wax components into water-insoluble hydroxy fatty acids (Sharma et al., 2020). Because these hydrolysis products are highly sticky, they can adhere to brush surfaces, especially within densely distributed filaments, making wax residues difficult to remove during routine cleaning. On waxing roller brushes, indigenous microorganisms, such as bacteria, yeasts, and molds, may shelter in wax residues or within the brush filaments, making them hard to sanitize.

Although some studies have investigated pathogen cross-contamination during waxing, few studies have evaluated the microbial safety associated with waxing roller brushes. Therefore, in this study, the persistence of LM on waxing roller brushes made with different materials and of different filament arrangement patterns with the presence of dried waxes, was investigated. In addition to laboratory-

based inoculation studies, a field study to better analyze the levels of the indigenous microbial population on waxing roller brushes in commercial packinghouses was conducted. The aim of the packinghouse sampling is to understand how native microbial populations change before and after cleaning and sanitation across the peak harvesting season. In the meantime, the efficiency of different cleaning and sanitation protocols in removing waxes and decontaminating microorganisms was investigated to optimize the cleaning and sanitation results, thereby mitigating microbial safety risks associated with waxing roller brushes.

Research Methods and Results

Methods

Study 1: Survival of *L. monocytogenes* and *E. faecium* on waxing roller brushes

Bacterial strains and inoculum preparation

A five-strain cocktail of rifampin-resistant LM was used for this study. In addition, the rifampin-resistant variant of EF NRRL-B2354 was used as the surrogate due to laboratory safety concerns when evaluating the impact of brush patterns on the attachment and survival of bacteria. For this experiment, approximately 15 Liters of bacterial suspension were needed to immerse brush coupons and the use of large volume of pathogen suspensions could pose safety concerns to laboratory personnel. Therefore, for the brush pattern impact evaluation, EF was used. To prepare the LM cocktail or EF suspension, the frozen stock of each strain was first streaked on tryptic soy agar (TSA) supplemented with rifampin at 50 µg/mL (TSAR) and incubated overnight at 37 °C. A single colony was then transferred into 10 mL of tryptic soy broth (TSB) and incubated at 37 °C for 24 h. On the next day, a loopful (10 µL) of the overnight liquid culture was transferred into 10 mL of fresh TSB and incubated at 37 °C for additional 24 h. Two hundred fifty microliters of fresh liquid culture were then plated onto a TSAR plate and incubated at 37 °C for 24 h to form the bacterial lawn. The bacterial lawn was harvested by adding 5 mL of phosphate-buffered saline (PBS) to the plate, then scraping with an L-spreader. The resulting culture suspension (~10 log CFU/mL) was collected into a 15-mL centrifuge tube. The cocktail of LM was prepared by combining equal volumes of suspended bacterial lawn of each strain and then diluted before used for inoculation.

1.1 Impacts of brush materials and the presence of waxes on the survival of *Listeria monocytogenes* Brushes

Tufted brush coupons (4.5 × 8.5 cm, Power Brush, Oregon) with different brush filament materials—nylon and 50% horsehair/50% polyethylene (50/50)—were used. Brush coupons were further cut into quarters (brush quarters) at the UC Davis mechanic shop.

Inoculation and storage

When wax was not applied to brushes, the inoculum of LM cocktail was drop-inoculated (10 × 10 µL drops) onto brush quarters (nylon or 50/50) at ~8 log CFU/brush quarter. After drying overnight in the biosafety cabinet with airflow on at ambient conditions (21.1 °C ± 0.3 °C, relative humidity (RH) = 52.2% ± 3.3%), the dried inoculated brush quarters were individually packed in Whirl-Pak bags and stored at ambient temperature for 3 months. Samples were taken on Days 0, 3, 7, 14, 21, 28, 42, 56, 70, and 84 for LM recovery and enumeration.

Commercial carnauba-based wax was used to coat or saturate the brush quarters. To do so, brush quarters were placed in a total of ~4 L of liquid wax to ensure full submersion of the brush filaments in the liquid waxes. Brush quarters were kept in the liquid wax for 60 seconds and then dried in the

biosafety cabinet with airflow on under ambient conditions for 48 hours. They were then used for bacterial inoculation (**Figure 1**). The inoculation steps for waxed brushes followed the same steps as mentioned above. Two biological replicates were done with three technical replicates (n = 6).

Bacterial recovery and enumeration

At each point, 100 mL Dey-Engley (D/E) solution with 1% Tween 20 was used to recover the bacteria by a 3 min shake-rub-shake method (Wang et al., 2024). The generated suspension was diluted and plated onto non-selective agar, TSA, and selective agar, Modified Oxford Agar (MOX), both supplemented with 50 mg/mL rifampin (TSAR and MOXR) were used for enumerating LM. All plates were incubated at 37 °C for up to 48 h, and the limit of detection (LOD) was 2 log CFU/brush quarter. If the counts were below the LOD, enrichment was performed in TSB to detect LM.

1.2 Impacts of brush materials and filament arrangement patterns on the survival of microorganisms *Brushes*

Tufted brush coupons (12.1 × 8.1 cm) and spiral brush coupons (12.3 × 8.5 cm) made with different brush filament materials (nylon or 50/50) were used.

Inoculation and storage

Brushes were inoculated with the EF inoculum, a surrogate for LM (~8 log CFU/brush coupon), by dipping (submerging) and rolling the entire brush coupon in EF suspension for 1 min. The fully covered and soaked brush coupons were dried for 48 hours in a biosafety cabinet with airflow on at ambient conditions (21.1 °C ± 0.3 °C, RH = 52.2% ± 3.3%). After drying, the dried inoculated brushes were individually packed in Whirl-Pak bags and stored at ambient temperature for 3 months. Days 0, 5, 10, 30, 60, and 90 were selected as sampling points. Two biological replicates were done with three technical replicates (n = 6).

Bacterial recovery and enumeration

At each sampling point, 100 mL of PBS solution was used to recover the bacteria from the inoculated brush coupons by a 3 min shake-rub-shake method (Wang et al., 2024). The suspension was then plated onto TSAR for the enumeration of surviving EF. All plates were incubated at 37 °C for up to 24 h, and the LOD was 2 log CFU/brush coupon. If the counts were below the LOD, enrichment was performed in TSB to detect EF.

Study 2: Evaluate the changes of indigenous microbial populations on waxing roller brushes before and after cleaning and sanitation during harvest season

2.1 Facility sampling

Three commercial stone fruit packinghouses in California were sampled at the beginning, during, and the end of the harvest season in 2024 and 2025. In 2024, samples were collected in July, August, and September from three facilities (facilities A, B, and C). In 2025, one facility dropped from the study and a new facility joined. Thus, facilities A, C, and D were sampled in June, July, and August 2025.

During the sampling trips, samples from waxing roller brushes were collected before and after cleaning and sanitation. Two adjacent areas (each 10 cm × 10 cm) were sampled on each brush. One area was swabbed with a sponge pre-moistened with 10 mL of D/E broth, and the other area was sampled with a sponge pre-moistened with 10 mL of neutralizing buffer (World Bioproducts, WA). The swabbing process contained 10 vertical and 10 horizontal strokes (**Figure 2**). Six biological replicates of brushes were collected at each sampling point per facility. After sampling, sponges were packed with ice and transported to the laboratory within 24 hours.

2.2 Enumeration of indigenous microorganisms

Sponge samples pre-moistened with 10 mL D/E broth were used for culture-dependent analysis. To facilitate bacterial recovery, each sponge was submerged in 10 mL of additional D/E broth and smashed for 60 seconds. Plate Count agar (PCA), CHROMagarECC (ECC), and Dichloran Rose Bengal Chloramphenicol (DRBC) were used to enumerate total mesophilic bacteria, coliforms, and yeasts/molds, respectively.

2.3 Culture-independent analysis of indigenous microorganisms

Sponge samples pre-moistened with 10 mL neutralizing buffer were used for culture-independent analysis. To facilitate bacterial recovery, each sponge was submerged in 10 mL of additional PBS solution and smashed for 60 seconds. After that, 5 mL of each sample suspension was centrifuged at X 8,000 g for 10 min, and the formed pellet was treated with propidium monoazide (PMA, Biotium, CA) at a final concentration of 50 μ M. PMA treatment was performed to bind the DNA of dead cells and prevent it from amplification during the sequencing process. The PMA treatment process contained a dark incubation period at 30 °C for 15 min, vortexed every 5 min to allow PMA to permeate injured cells. After the treatment, remaining PMA was denatured by exposing the samples to a blue LED (λ = 465–475 nm) for 15 min at room temperature (Rolon et al., 2024). The PMA-treated samples were then used for DNA extraction with the Qiagen DNeasy PowerSoil Pro Kit according to the manufacturer's instructions. Qubit fluorometer was used to quantify the concentration of DNA.

High-quality DNA samples were used for library preparation using the QIAseq 16S/ITS Screening Panel kit, which targets the V3-V4 and ITS regions. The library sizes and concentrations were assessed using a bioanalyzer. The sequencing was carried out on the AVITI platform at the UC Davis Genome Center. DADA2, with default parameters, was applied in the QIIME2 pipeline to denoise the 16S rRNA and ITS raw FASTQ files. Taxonomic assignments were performed using the QIIME2 q2-feature-classifier plugin. Sequences classified as Archaea, Eukaryota, chloroplasts, or mitochondria will be excluded. Alpha and beta diversity were also analyzed in QIIME2. Alpha diversity was summarized using the Shannon index, while beta diversity was evaluated using distance metrics (Bray–Curtis). Mock Community (ZymoBIOMICS® Microbial Community Standard) was included as a positive control and processed alongside samples through DNA extraction, library preparation, and sequencing step.

Study 3: Optimize and validate the cleaning and sanitation protocols to effectively clean and decontaminate waxing roller brushes

3.1 Brush cleaning

Materials

Tufted nylon and 50/50 brush quarters were selected. In addition, brush filaments were cut for microscopic examination. Commercial carnauba-(CN), mineral-oil-(MO), and vegetable-oil-(VO) based waxes were used to check the efficacy of different cleaning and sanitation protocols. An alkaline cleaner (Sunrise Environmental Scientific, NV), a neutral cleaner (Cascade, Toronto, ON), and deionized (DI) water were tested.

Coating brush filament and coupons with wet wax or dried wax

Loose brush filaments and brush quarters were coated with wet wax or dried wax. In the coating process for loose filaments, the filaments were fully immersed in liquid wax (CN-based, MO-based, or VO-based) to ensure complete saturation. For brush filaments requiring dried wax coatings, the wax-coated filaments were transferred to a weighing boat for drying in ambient conditions. The preparation of wax coated brush quarters followed the same protocols described above.

Cleaning of waxed brush filaments and quarters

For brush filaments coated with wet wax, the filaments were rinsed directly with 100 mL of DI water, repeated for five times, and then dried at ambient temperature. For brush filaments coated with dried wax, the waxed filaments were soaked in a 45 mL bath of alkaline cleaner (pH = 12.55), neutral cleaning agent (pH = 8.56), or DI water for 15 min. Then, the filaments were rinsed with 100 mL of DI water and air-dried.

For brush quarters coated with wet wax, they were rinsed directly with 2 L of water at a flow rate of 60 L·s⁻¹ with a lab scale high-pressure washer gun equipped with a 0° nozzle (1.0 mm diameter). After rinsing, the brush quarters were air-dried under ambient conditions until a constant mass was achieved. For brush quarters coated with dried wax, they were either directly rinsed with 2 L of water (60 L·s⁻¹) or treated with a chemical treatment or a combination of chemical and physical treatments to soften the dried waxes. These treatments included **1**) spraying with 25 mL of alkaline cleaner, **2**) soaking in 400 mL of alkaline cleaner at 26 °C, **3**) ultrasound treatment (Branson 1510 ultrasonic cleaner, Emerson Electric, CT) in 400 mL of alkaline cleaner at 26 °C, **4**) soaking in 400 mL of alkaline cleaner at 50 °C, and **5**) ultrasound treatment in 400 mL of alkaline cleaner at 50 °C. All softening treatments lasted 15 min, and then the brush quarters were rinsed with 2 L of water (60 L·s⁻¹) and air-dried under ambient conditions until a constant mass was achieved.

3.2 In-lab cleaning and sanitation experiment and pilot plant validation

Sanitizer preparation

Based on the results obtained from 3.1, alkaline cleaner which was prepared by mixing detergent with Milli-Q water at a 1:8 (v/v) ratio was used to conduct cleaning and sanitation experiments. PAA at 500 ppm (32% C₂H₄O₃ stock, Spectrum Chemical, CA) and chlorine (at 200 ppm, 6% sodium hypochlorite [NaClO], WAXIE, germicidal ultra bleach) were prepared for sanitation.

Preparation of wax coated brush quarters and inoculation

The application of waxes on brush quarters as well as the inoculation of waxed brush quarters followed the procedures described above.

In-lab cleaning and sanitation treatments

Five cleaning and sanitation protocols included **1**) cleaning with water only, **2**) cleaning with alkaline cleaner only, **3**) alkaline cleaner followed by water rinsing, **4**) alkaline cleaner followed by water rinsing and sanitizing with PAA, and **5**) alkaline cleaner followed by water rinsing and sanitizing with chlorine.

For wet waxes, inoculated quarters were cleaned by rinsing with 100 mL of Milli-Q water or alkaline cleaner. For dried waxes, inoculated brush quarters were immersed with filaments facing downward in 1 L of Milli-Q water or alkaline cleaner and soaked for 15 min to soften dried waxes. After cleaning with alkaline cleaner, brush quarters were rinsed with ~100 mL of Milli-Q water using a 48-oz hand-pump sprayer (Chapin International 1046, Batavia, NY) held at a distance of ~3 cm. For the sanitation step, 3 mL of PAA or chlorine was applied to the brush quarter surfaces by spraying the sanitizer solutions from ~3 cm distance, followed by a 15-min contact time.

Pilot plant validation

A cleaning-in-place (CIP) cleaning and sanitation protocol, including the use of alkaline cleaner (1:8 v/v) and PAA (500 ppm), was evaluated during the pilot plant validation. Ten roller brushes on the brush bed were divided into two groups (five front brushes near the entrance and five back brushes near the exit). Each brush was divided into four sampling sections. Because each brush contained a

hi-lo trim profile in the center, sampling locations were alternated to make sure the cleaning and sanitation efficacy was tested on different types of surfaces.

To prepare inoculated and wax-coated roller brushes at the pilot plant, wax was applied at 1.0 gallon per hour (gph) through a top-mounted spray nozzle (waxer) positioned 28 cm above the brush surface at the geometric center of the waxing bed. After that, approximately 100–150 uninoculated fruits ran through the waxing bed for 2 min with the waxer operating and brushes rotating at the default operating speed to thoroughly coat the brushes with wax. After that, the waxed uninoculated fruits were removed and the spray nozzle was turned off, leaving the roller brushes running. Then, ~100–150 inoculated fruits ran through the waxing bed for 2 min to transfer EF from fruit surfaces to the brushes. In this case, the inoculation of waxing roller brushes was completed. The preparation of inoculated fruit followed the protocol developed by Sheng et al. (2017).

Sampling and analysis

After the cleaning and sanitation, each brush surface unit (15 cm × 14 cm) was swabbed in both horizontal and vertical directions for 10 passes to ensure full coverage. Polyurethane sponges pre-wetted with 10 mL D/E broth were used for swabbing. After sampling, sponges were placed into sterile sampling bags, stored in an insulated foam box with ice packs, and transported to the laboratory for microbial analysis within 24 h. For laboratory experiments, brush quarters (control and post-treatment) were collected into 1627-mL (55-oz) Whirl-Pak filter bags containing 100 mL of D/E broth with 1% Tween 20 and processed using a shake-rub-shake protocol for 3 min. All sample suspensions were diluted in PBS as needed and spiral-plated in duplicate onto TSAR and Slanetz and Bartley (S+B) agar. Plates were incubated at 37 °C for 24 h (TSAR) or 48 h (S+B).

Statistical analysis

For the cleaning and sanitation trials, one-way ANOVA followed by Tukey's multiple comparison test or Welch's t test was used to evaluate differences among treatments or between different media, with significance defined as $P < 0.05$. Statistical analyses were performed using GraphPad Prism software (version 10, GraphPad Software, Inc., CA).

Results

Study 1: Survival of *L. monocytogenes* and *E. faecium* on brushes

Impact of filament materials on the survival of *L. monocytogenes*. The initial inoculation levels of LM on both tufted-nylon and tufted-50/50 brush quarters were around 7 log CFU/brush quarter. After 42 days of storage, the population of LM decreased to ~3.5 log and ~6 log CFU/brush quarter on tufted-nylon and 50/50 brush quarters, respectively. Over the next 42 days of storage, the population of LM on tufted nylon and 50/50 brushes gradually declined to ~2 log and 5 log CFU/brush quarter, respectively. These results indicated filament material could impact the survival of LM on tufted brush quarters.

Impact of dried wax on the survival of *L. monocytogenes*. For waxed brushes, the reduction of LM on tufted nylon and 50/50 brush quarters was similar. On both waxed brushes, the population of LM decreased by ~5 log CFU/brush quarter by Day 56. On 50/50 brushes, the LM population remained at ~2.5 log CFU/brush quarter from Day 56 to Day 84. On nylon brushes, bacterial population dropped below the LOD (2 log CFU/brush quarter) on Day 70, but with enrichment positive. Together, these results suggest that dried wax may outweigh material differences in survival, indicating that its presence was the primary determinant of LM persistence on waxed tufted brushes.

Impact of brush pattern and materials on the survival of *E. faecium*. After 90 days of storage, EF decreased by ~1.5 log CFU/brush coupon on spiral nylon brushes and by ~1.7 log CFU/brush coupon on tufted nylon brushes. For 50/50 brushes, the material was likely creating more protected niches, so bacterial cells died off more slowly. EF declined by ~1 log CFU/brush coupon for spiral brushes and by 0.5 log CFU/brush coupon for tufted brushes. These results indicate that brush patterns and material collectively impact the survival of EF.

Study 2: Evaluate indigenous microbial composition on waxing roller brushes in packinghouses

Enumeration of microorganisms sampled from waxing roller brushes. Three packinghouses participated in the 2024 sampling season, and three visits were done for each facility across the harvest season. The total aerobic plate counts (APC) ranged from approximately 6 to 8 log. For two of the facilities, no difference was observed before and after cleaning and sanitation. During the harvest season, the intensive cleaning out-of-place led to approximately 2-4 log reduction for APC and coliform. With this intensive cleaning out-of-place practice, the last sampling trip of the year showed lower APC, coliform, and fungal counts, even though it was at the end of the harvest season. During the 2025 sampling season, the initial APC, coliform, and fungal counts among all participating facilities were lower than in 2024, ranging approximately 2-4 log. All three facilities used alkaline cleaner. By the end of sampling season, the APC, coliform, and fungal counts ranged from 3-6 log.

Alpha and Beta diversity outcomes and pipeline validation using a mock community. All genera expected in the ZymoBIOMICS Microbial Community Standard were recovered by the workflow, supporting the robust performance of the pipeline (DNA extraction → library preparation → 16S/ITS sequencing → bioinformatics analysis). In addition, all expected genera were recovered in both PMA-treated and PMA-untreated mock samples, and community profiles were similar, suggesting that the PMA treatment only inactivated the dead cell's DNA and did not influence the sequencing of live cells.

Alpha diversity was evaluated using the Shannon index, and the results indicated that richness and evenness did not differ significantly between samples collected before and after cleaning and sanitation. Beta-diversity analyses of bacterial community composition indicated significant compositional differences between before and after cleaning and sanitation. Together, these results suggest that cleaning and sanitation altered which taxonomic group dominated community composition rather than changing overall diversity (richness).

Study 3: Optimize and validate the cleaning and sanitizing protocols to effectively remove wax and microorganisms from brushes

Physical properties of waxes. After 5 days of air exposure, CN-based wax solidified into chunks upon drying, whereas MO-based wax remained sticky. VO-based wax was retained in oily form. When those waxes were coated onto the nylon brush filaments, the solvent in the CN- and MO-based waxes evaporated within 4 h, whereas the solvent in the VO-based wax took nearly 50 h. On 50/50 brush filaments, solvent evaporation took 6 h for CN-based wax, 10 h for MO-based wax, and approximately 50 h for VO-based wax. These differences in solvent evaporation were mainly driven by differences in wax pick-up, which depended on both filament morphology and wax properties. Overall, 50/50 filaments consistently retained more wax CN- and MO-based wax than nylon filaments.

Cleaning efficiency of waxed brush filaments. For wet wax removal of brush filaments, thorough rinsing effectively cleaned the filament surface. This method removed over 99% of wet waxes, regardless of the wax on both filament types (nylon and 50/50). For dried waxes, direct water rinsing removed only ~23% of the dried CN-based wax from nylon fibers. The wax removal rate of soaking CN-based wax-coated nylon filaments in alkaline cleaners, neutral cleaners, and DI water was ~99%, 64%, and 24%,

respectively. When nylon filaments were coated with dried MO-based wax, wax removal was more efficient. Soaking in either alkaline cleaners or neutral cleaners for 15 min achieved wax removal rates of ~98% and 97%, respectively. When nylon filaments were coated with VO-based wax, the wax removal rate was highly efficient across all treatment types. Soaking in alkaline cleaners, neutral cleaners, and DI water all achieved ~98% removal. The cleaning efficiency of the three waxes on 50/50 brush filaments was similar. CN-based wax was the most difficult to remove, and alkaline cleaners achieved the highest wax-removal rate compared with neutral cleaners and water.

Cleaning efficiency of waxed brush quarters. For brush quarters with one layer of dried wax, pressurized water rinsing alone removed only 1.2% and 11.7% of the dried waxes from nylon and 50/50 brush coupons, respectively. With the application of physical and chemical washing for one cycle, approximately 32, 67, 80, 74, and 85% of the dried CN-based wax was removed from nylon brush quarters using spray, 26 °C soak, 26 °C ultrasound (US) treatment, 50 °C soak, and 50 °C US, respectively; and for 50/50 brushes, around 62, 65, 70, 65, and 83% of the dried CN-based wax was removed by spray, 26 °C soak, 26 °C US, 50 °C soak, and 50 °C US, respectively. After two washing cycles, the wax removal rate exceeded 85% for both brush types under 26 °C US treatment and 50 °C US treatment. When five layers of wax were coated on both brushes, after three washing cycles, both 26 °C US treatment and 50 °C US treatment achieved around 90% wax removal rate on nylon and around 80% wax removal rate on 50/50 brushes. The results showed the synergistic effect between physical forces (ultrasound or elevated temperature) and chemical forces (alkaline cleaners) helped improve cleaning efficiency for nylon and 50/50 brush quarters.

In-lab and pilot-plant validation. When waxes were still wet, cleaning and rinsing achieved ~3-4 log reductions of EF while the subsequent sanitation with chlorine or PAA provided limited additional reductions. For dried-wax brushes, cleaning and sanitation efficacy was significantly reduced, with < 1 log reduction after detergent soaking, rinsing, and sanitation. Pilot-plant validation further confirmed a ~3-4 log reduction of EF on brushes when cleaning and sanitation was done when waxes were still wet with CIP procedures.

Outcomes and Accomplishments

- The survival of LM on unwaxed and waxed brushes made of nylon and 50/50 materials was investigated over 84 days of storage.
- The survival of EF on spiral- and tufted-pattern brushes made of nylon and 50/50 materials was investigated over 90 days of storage.
- Field sampling in stone-fruit packinghouses was conducted in 2024 and 2025 to evaluate the impact of sampling time and cleaning and sanitation practices on microbial composition. Both culture-dependent and culture-independent analyses were done.
- The findings were shared with the fresh fruit packinghouses through presentations, field visits, and multiple meetings.
- Cleaning protocols for effectively removing wet and dried waxes from brush filaments and brush quarters were optimized.
- A pilot study at the Lindcove Research and Extension was completed to validate sanitation protocols for removing microorganisms from waxing roller brushes.
- A manuscript with the objective to investigate the cleaning efficiency of different cleaners on waxed brush filaments and brush coupons was published in a peer-reviewed journal.

APPENDICES

Publications and Presentations

Publications

Jiahan Zou, Chih-Rong Lee, Sidd Dala, Luxin Wang, Gang Sun. (2025). Efficient cleaning protocols for waxing roller brushes to mitigate food safety concerns in packing houses. *ACS Food Science & Technology*, 5(6), 2317-2328. <https://doi.org/10.1021/acsfoodscitech.5c00174>

Note: Three more manuscripts for publication in scientific journals are in preparation.

Dissemination of research findings to stakeholders

2024 June. The PI and postdoctoral fellow attended the CPS Research Symposium poster session and presented the work to date.

2024 November. The PI, postdoctoral fellow, and Ph.D. student attended the CPS virtual site visit and gave a presentation on annual progress updates.

2025 June. The PI and postdoctoral fellow attended the CPS Symposium poster session and gave a 5-minute presentation.

2025 September. The PI attended the Citrus industry annual meeting and presented the results of this study to citrus growers and processors.

2025 November. The PI attended the CPS virtual site visit and gave a presentation on annual progress updates.

Presentations

2024 July 14-17. Zhuo Chen and Luxin Wang. Survival of *Listeria monocytogenes* on Roller Brushes Made with Different Materials (P1-125). International Association for Food Protection (IAFP) Conference 2024, Long Beach, California.

2025 May 6. Zhuo Chen, Yucen Xie, Tianyi Tu, Yuqin Zhang, Elise Kim, and Luxin Wang. Impact of Filament Materials and Wax on the Survival of *Listeria monocytogenes* on Commercial Waxing Roller Brushes. Northern California Institute of Food Technologists (NCIFT) Conference 2025, Pleasanton, California.

2026 September 13. Luxin Wang. Characterization and mitigation of food safety risks associated with waxing roller brushes. Visalia, CA.

Budget Summary

This project was awarded \$407,384 in research funds. The majority of funds have been spent as planned to support the associate specialist, graduate students, and undergraduate students who worked on this project, and to purchase supplies for this study.

Figures

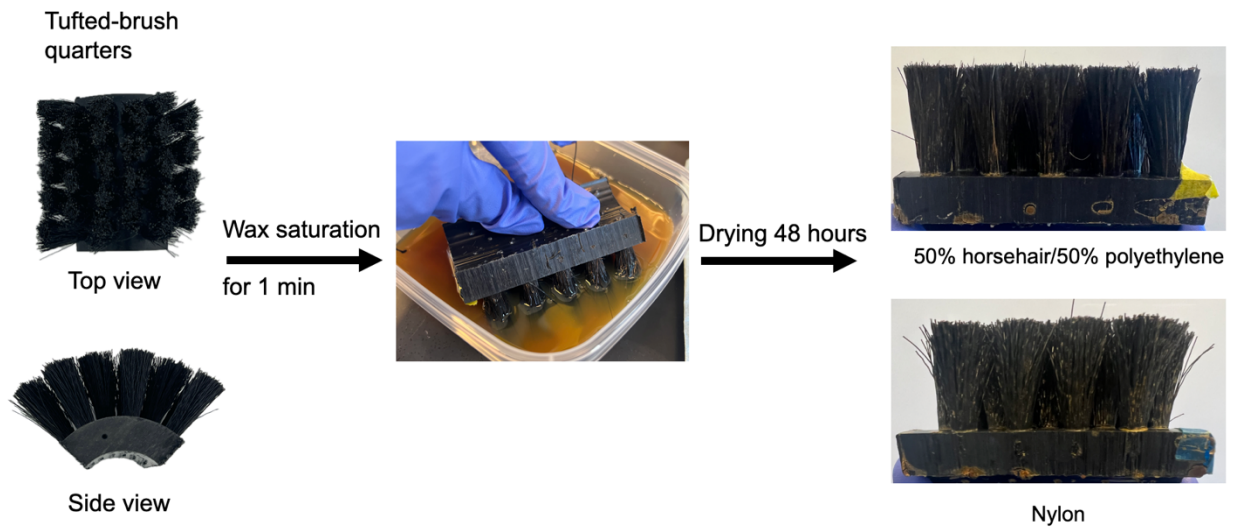


Figure 1. Workflow for wax saturation and drying of tufted-brush quarters (nylon vs. 50/50)

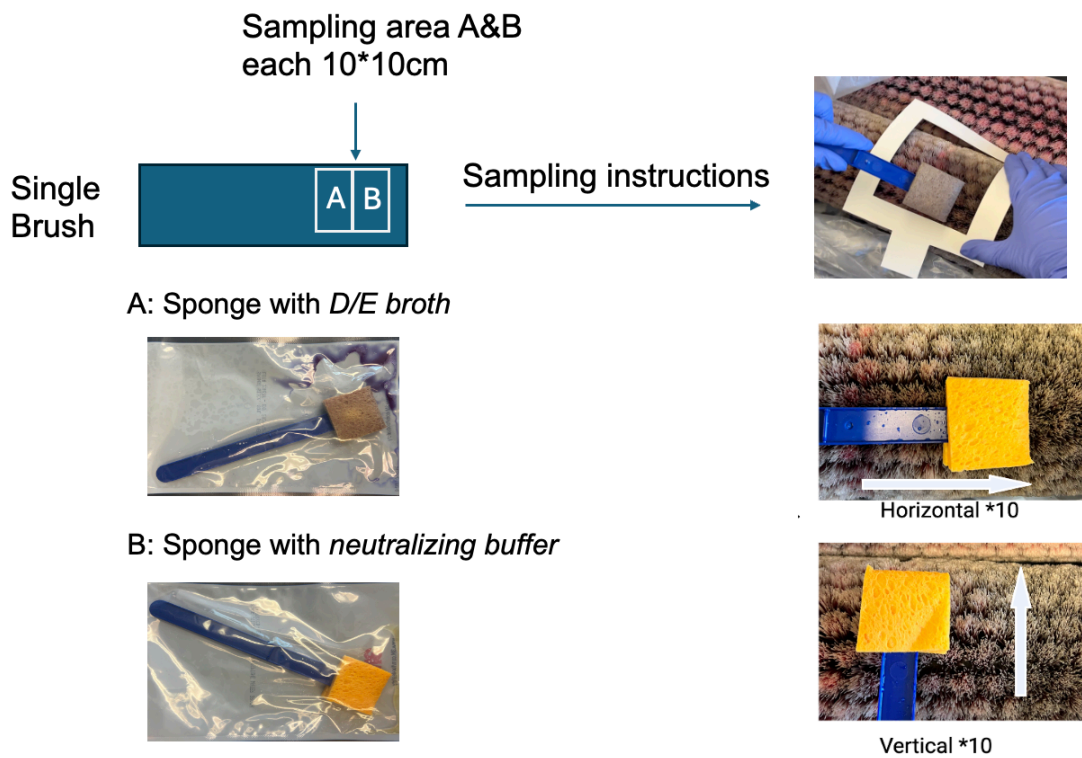


Figure 2. Brush surface sampling design (areas A and B) and sampling instructions (10 horizontal + 10 vertical strokes)

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