CPS 2023 RFP – FINAL PROJECT REPORT

Project Title:
Synergistic antimicrobial activity of food-grade compounds in wax coatings on fruits during wax drying

Project Period:
January 1, 2024 – December 31, 2025 (extended to January 31, 2026)

Principal Investigator:
Nitin Nitin, PhD
University of California, Davis
Department of Food Science and Technology
One Shields Avenue
Davis, CA 95616
T: 530-752-6208
E: nnitin@ucdavis.edu

Co-Principal Investigator:
Meijun Zhu, PhD
Washington State University
School of Food Science
T: 509-335-4016
E: meijun.zhu@wsu.edu

Objectives:

1. Assess the synergistic interaction of LAE (ethyl lauroyl arginate) and other food-grade compounds or extracts with mild heat to achieve rapid inactivation of bacteria inoculated in a wax suspension.
2. Assess the role of synergistic treatment (optimal combination of food-grade compounds in a wax coating and mild heat identified in Objective 1) in the inactivation of the pathogens inoculated in a wax composition on the surface of apples and citrus fruits.
3. Measure the influence of synergistic treatment in the inactivation of the pathogens inoculated on the surfaces of apples and citrus, including the stem and calyx regions.
4. Evaluate the influence of the optimal synergistic treatments identified in Objectives 2 and 3 on the quality (including color), microbial load (endogenous), and shelf life of fruit during storage.

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FINAL REPORT

Summary of Findings and Recommendations

Summary of Findings:

- **Strong inhibitory activities of carnauba-based wax products** (Objective 1)
  - Carnauba-based wax formulations demonstrated intrinsic activity to inactivate bacterial cells in wax suspension, with morpholine-supplemented waxes showing stronger inhibition than non-morpholine formulations.
  - Gram-positive bacteria were more susceptible than Gram-negative bacteria.
  - Heating wax formulations to 40–50°C further enhanced the inhibitory activity.

- **Strong synergistic effects were achieved with olive pomace extract (OPE) or propyl gallate (PG) combined with mild heat in wax formulations** (Objectives 2 & 3)
  - On orange skins and whole oranges, OPE- or PG-supplemented wax combined with 55°C drying achieved greater than 3-log reductions within 2–5 minutes, including reductions on fruit surfaces, including stem and calyx regions.
  - Strong synergistic activities were observed on citrus but were limited in apple-specific wax formulations.
  - However, heated OPE alone achieved greater than 4-log reductions on apple peels, indicating a promising alternative strategy for apples. Based on this approach, sequential OPE treatment resulted in 2-log inactivation of bacteria on dip-inoculated apples. The reduction was lower as compared to oranges but significantly improved with the sequential treatment process.
  - Sequential OPE application to apples resulted in ~ 1.1 log CFU reduction in total aerobic plate counts and a ~0.5 log CFU reduction in yeast and mold (YM) counts, compared to ~0.5 log CFU and ~0.3 log CFU reductions in total aerobic plate counts and YM counts, respectively, achieved with the 55 °C water treatment followed by wax coating.

- **Improved fruit quality and shelf life** (Objective 4)
  - Oranges treated with OPE-supplemented wax combined with mild heat showed slower microbial buildup during 4 weeks of storage at 4°C compared to control oranges with standard wax coating.
  - Oranges treated with OPE-supplemented wax combined with mild heat showed reduced discoloration (about 2-fold lower ΔE^*) and reduced weight loss (about 1.9-fold lower) during 8 weeks of storage at 4°C compared to controls.
  - During 12 weeks of storage, total aerobic plate counts and YM counts on OPE-treated apples remained relatively stable. In contrast, other treatment groups exhibited a general reduction in total aerobic plate counts and a slight increase in YM populations over the storage period.

Recommendations for industry:

- Evaluate OPE- or PG-supplemented carnauba wax combined with 50–55°C drying in commercial citrus operations.
- For apples, consider heated plant extract pre-treatment approaches prior to wax coatings.

Abstract
This project aims to enhance the inactivation of bacteria on wax coatings and fruit surfaces during the wax coating drying process. The enhanced inactivation of bacteria on wax coatings and fruit surfaces was achieved using a synergistic antimicrobial approach proposed in this research. This synergistic inactivation of bacteria is based on the combined effect of selected GRAS compounds added to commercial wax compositions, with mild heat-assisted drying of wax coatings. Overall, this research was motivated by the needs of the fresh fruit industry to improve the safety of fresh fruits during postharvest processing. This research project focuses on both citrus and apple fruits. The specific goals of this project were to (a) assess the synergistic interaction of LAE (ethyl lauroyl arginate) and other food-grade compounds or extracts with mild heat to achieve rapid inactivation of bacteria inoculated in a wax suspension; (b) evaluate the role of the synergistic treatment in the inactivation of pathogens inoculated in a wax composition after coating on citrus fruits and apples; (c) demonstrate the efficacy of the synergistic treatment in inactivating pathogens inoculated on the surface of apples and citrus fruits; and (d) evaluate the influence of the optimal synergistic treatments on the quality, microbial load, and shelf life of fruits. This research demonstrated inactivation of bacteria in wax coatings, including in suspension and on fruit surfaces, and demonstrated the potential to reduce cross-contamination risks from wax compositions and brushes and improve fresh fruit safety by reducing the bacterial load on the fruit surface.

Background
Apples and citrus fruits are coated with food-grade waxes as a final postharvest processing step to improve shelf life, enhance glossiness, and minimize moisture and weight loss during storage and distribution [1–3]. In apple and citrus industries, carnauba-based natural waxes are commonly used as finishing waxes [3,4]. These wax coatings are sold under commercial labels, e.g., PrimaFresh 360 and PrimaFresh 660 for the apple industries and Natural Shine 960 and Natural Shine 965 for the citrus industries. Although these wax coating compositions are proprietary, these compositions possibly include wax microemulsions stabilized in water with fatty acids, in the presence of ammonia, and other food grade components [5]. In addition, citrus industries also use a wax coating to store the fruit [6]. In many industries, particularly citrus and apple industries, wax coatings are air-dried using hot-air at temperatures between 45-60°C for up to 6 min duration [7–9]. This range of temperature and time represents the typical temperature-time range used in the apple and citrus industries. Previous research, including research supported by CPS and the Washington Tree Fruit Research Commission, has evaluated the role of wax coatings on microbial loads on the fruit surface. The results of these studies indicate: (a) Wax materials derived from natural sources can have a relatively moderate level of microbial loads (10^3–10^5 cells/mL), including fungi and bacteria [10]; (b) Results from the challenge studies have shown that both *Listeria* and *Salmonella* strains introduced in wax coatings could survive during the coating process, including mild heat, and can significantly enhance the risks of cross-contamination, possibly through the contamination of wax brushes [11]; (c) Wax coatings have a limited impact on the inactivation of microbes on the fruit surface. In some cases, studies have reported an increase in microbial survival in wax-coated surfaces [12]. Overall, these results indicate the limited inactivation of hazardous microbes during the wax coating process.

Wax coating is the last step in the postharvest processing of many fruits. Thus, preventing cross-contamination from the wax formulation or the processing surfaces such as wax brushes could significantly reduce food safety risks [11,14]. Furthermore, reducing the microbial load, including pathogens contaminated on a fruit surface, is vital for enhancing the microbial safety of fresh fruits [15]. Therefore, this research is timely, as previous studies [11,14] have illustrated that wax coatings can harbor microbes, including pathogens. However, the current process and composition of wax coatings have a limited impact on the survival of microbes/pathogens. Furthermore, various additives such as

essential oils [16], extracts [17], and other food-grade compounds in wax coatings have shown potential to improve the preservation of fruit with extended storage but have not demonstrated success in reducing cross-contamination risks and microbial load during the fruit processing. Thus, this research was motivated by the needs of the fresh fruit industry to improve the safety of fresh fruits during postharvest processing. This research focuses on using wax coatings modified with generally recognized as safe (GRAS) compounds or plant-derived extracts to achieve synergistic inactivation of bacteria both in the wax coating and on the fruit surface during the wax coating drying process. The success of this project will be reduced cross-contamination risks from wax compositions and brushes and improved fresh fruit safety by reducing the bacterial load on the fruit surface. This synergistic inactivation of bacteria is based on the combined effect of selected GRAS compound with mild heat. The mechanisms for this synergistic activity may include enhanced membrane damage, synergistic induction of oxidative stress, suppression or inactivation of key enzymes and damage to protein/DNA and lipid structures [18,19]. In addition, the GRAS compound or edible plant extracts are selected to reduce barriers to the translation of the research to industry applications and provide cost-effective solutions that the industry can implement.

Research Methods and Results

Objective 1. Assess the synergistic interaction of LAE (ethyl lauroyl arginate) and other food-grade compounds or extracts with mild heat to achieve rapid inactivation of bacteria inoculated in a wax suspension.

a. Selection and preparation of wax

Three types of commercial wax products were kindly provided by JBT Corporation. These include two carnauba-based waxes, with (SF200) and without (EF100) morpholines, and a vegetable oil–based wax (SF206). The wax formulations were prepared by following the supplier's instruction, and SF206 was diluted by 17.5-fold with deionized water. The pH values of the selected wax products were 9.5 (EF100), 8.6 (SF200), and 7.6 (SF206).

b. Inhibitory activities of the commercial wax against bacterial pathogens

The inhibitory activities of the selected wax formulations were tested against *Escherichia coli* O157:H7, *Listeria innocua*, and *Enterococcus faecium* (**Figure 1**). Overall, strong antimicrobial activities were exerted from carnauba-based wax with morpholines (SF200), followed by carnauba-based wax without morpholines (EF100). Among tested bacterial strains, *E. faecium* showed the highest rate of inactivation in both EF100 and SF200, followed by *L. innocua* and *E. coli* O157:H7. In contrast, vegetable oil-based wax (SF206) only showed mild inhibitory activity against the Gram-positive strains (*E. faecium* and *L. innocua*), and no significant inhibitory activity was observed against the Gram-negative strain, *E. coli* O157:H7. These results indicate that carnauba-based wax formulations, especially the one containing morpholines, can exert strong antimicrobial activities against bacterial cells, and Gram-positive bacteria showed higher susceptibility than Gram-negative bacteria against tested wax products.

c. Antimicrobial activities of heated wax (40, 45, and 50°C)

The antimicrobial activities of the heated wax formulations were evaluated against *E. coli* O157:H7 (Gram-negative) and *L. innocua* (Gram-positive). As suggested by the CPS advisory committees, a mild temperature range (40–50°C) was selected and tested to minimize the possible thermal damage to the fresh produce. Overall, significantly enhanced inhibitory activities were exhibited for EF100 and SF200 when wax products were heated over 40°C, and stronger inhibitory activities were observed as the temperature of the wax increased from 40°C to 50°C (**Figure 2**). In contrast, SF206 showed no

significant inhibitory activity against *E. coli* O157:H7, regardless of the temperature. In addition, both heated and non-heated wax treatments showed stronger inhibitory activities against *L. innocua* compared to *E. coli* O157:H7. Heated SF200 showed the strongest inhibitory activities, followed by heated EF100 and SF206, against both tested bacteria. Based on the results, 40°C was selected as a mild temperature for further combined treatment with food-grade antimicrobials in wax formulations.

d. Synergistic antimicrobial activities of lauric arginate (LAE) with mild heat in SF206

The synergistic antimicrobial activities of LAE and mild heat (55°C) were evaluated against *E. coli* O157:H7 and *L. innocua* cells in SF206 (**Figure 3**). The combined treatment of LAE and mild heat (55°C) resulted in enhanced antimicrobial activity against *L. innocua* in SF206, achieving >5-log reductions within 10 min. Interestingly, mild heat alone also exhibited strong antimicrobial activity against *L. innocua* in SF206, resulting in >4-log reductions within 10 min. However, only limited antimicrobial activities were exerted by the combined treatment against *E. coli* O157:H7 in SF206.

e. Synergistic antimicrobial activity of olive pomace extract (OPE) and mild heat (40°C) in wax products

The synergistic antimicrobial activity of OPE (1.0 mg GAE/mL) and mild heat (40°C) was evaluated in wax formulations. Overall, significant synergistic activity was observed with the combined treatment of OPE and 40°C in wax products against the tested bacteria (**Figure 4**). For example, the combined treatment of OPE and 40°C resulted in significantly enhanced antimicrobial activity against *E. coli* O157:H7 across all tested wax products (EF100, SF200, and SF206), compared to the controls (wax, wax + 40°C, or wax + OPE), within 10 min of treatment (**Figure 4a**). In addition, strong synergistic antimicrobial activity was exerted against *L. innocua* in SF206, and ca. 5-log reduction of *L. innocua* was achieved within 1 min, whereas no significant reduction of *L. innocua* population was achieved with the controls (SF206, SF206 + 40°C, or SF206 + OPE) (**Figure 4b**). These results suggest that strong synergistic antimicrobial activity could be induced by applying OPE and 40°C in commercial wax products.

f. Synergistic antimicrobial activity of gallate family (GF) and mild heat (40°C) in wax products

Among the phenolic components, gallic acid (GA, gallate) was identified as one of the major compounds in olive pomace extract (OPE). Therefore, the potential synergistic antimicrobial activities of GA and its derivatives (methyl gallate [MG], ethyl gallate [EG], propyl gallate [PG], and octyl gallate [OG]) in combination with mild heat (40°C) were evaluated against *E. coli* O157:H7 and *L. innocua* in wax products (**Figure 5**). Overall, significant synergistic activities were exerted by the combined treatment of gallate family (GF; 5 mM) and mild heat, showing stronger activities against *L. innocua* than *E. coli* O157:H7. GF compounds with shorter hydrocarbon chains (except GA) exhibited stronger activities than those with longer chains. The combined treatment exhibited stronger antimicrobial activities in the carnauba oil–based wax products (EF100 and SF200) than in the vegetable oil–based wax product (SF206). However, no significant antimicrobial activities were shown by the tested combinations against *E. coli* O157:H7 in SF206.

Objectives 2 & 3. Assess the role of synergistic treatment (optimal combination of food-grade compounds in a wax coating and mild heat identified in Objective 1) for the inactivation of the pathogens inoculated in a wax composition on the surface of apples and citrus fruits. Measure the influence of synergistic treatment in the inactivation of the pathogens inoculated on the surfaces of apples and citrus, including the stem and calyx regions.

a. Inhibitory activities of the commercial wax on orange skin

The inhibitory activities of the carnauba-based wax coating (EF100 and SF200) were tested on orange skins using three different approaches. In the first experimental setup, *L. innocua* cells were

spread-inoculated on orange skin before applying the wax coating (10 μL/cm^2). In the second experimental setup, the bacterial cells were co-inoculated with wax formulations on orange skin. In the third experimental setup, the bacterial cells were inoculated on orange skin after applying the wax coating onto orange skin. For a control sample, bacterial cells were inoculated on orange skin and air-dried for 30 min without applying wax coating. The bacterial population of the control was ca. 5.4 log CFU/cm^2. Overall, much weaker antimicrobial activities were observed when the wax coating was applied on orange skins compared to the previous results tested in the wax formulations (**Figure 6**). For example, EF100 did not show any significant decreases in the bacterial populations across all three experimental scenarios compared to those of the control. However, SF200 still resulted in significant inhibitory activities, and ca. 0.8-, 1.5-, and 3.1-log reductions were observed when bacterial cells were pre-, co-, and post-inoculated on orange skins, respectively.

b. Synergistic antimicrobial activities of selected antimicrobial compounds and dry heat on orange skin

OPE and PG were selected for further *in vivo* tests on orange skins due to their antimicrobial activities and/or generally recognized as safe (GRAS) status approved by the U.S. Food and Drug Administration (FDA). The synergistic antimicrobial activities of OPE (1.0 mg GAE/mL) or PG (5 mM) and dry heat (55°C) were evaluated in EF100 against *E. coli* O157:H7 cells on orange skins (**Figure 7**). The combined treatment of OPE/PG and dry heat exerted strong synergistic activities on orange skins and achieved >3-log reductions of pre-inoculated *E. coli* O157:H7 cells within 2 min of drying. In contrast, the bacterial cells treated with EF100 and dry heat without supplementation of antimicrobial compounds resulted in ca. 1.6- to 2.3-log reductions of *E. coli* O157:H7. These results indicate that combined treatment of OPE and dry heat can achieve synergistic inactivation of bacterial cells in carnauba-based wax formulation applied onto orange skins.

c. Synergistic antimicrobial activity of OPE and dry heat on whole orange

The synergistic antimicrobial activities of OPE (1.0 mg GAE/mL) and dry heat (55°C) were evaluated in EF100 against *E. coli* O157:H7 on whole oranges (**Figure 8**). The combined treatment of OPE and dry heat exerted strong synergistic activities on whole oranges and achieved ca. 2.9-, 4.0-, and 2.0-log reductions of *E. coli* O157:H7 cells pre-inoculated on the side, stem (top), and calyx (bottom) of whole orange fruits, respectively, whereas those treated with EF100 and dry heat without OPE resulted in ca. 1.3-, 2.6-, and 1.6-log reductions, respectively. These results indicate that supplementation of OPE in the wax formulation synergistically enhanced the inactivation of bacterial pathogens contaminated on whole orange fruits.

d. Limited synergistic antimicrobial activity of PG and dry heat on apple peels and whole apple fruits

The potential synergistic antimicrobial activity of PG (5 mM) in combination with mild heat (55°C) against *L. innocua* on apples was further evaluated with carnauba-based wax formulations, PrimaFresh 360 (PF360; with morpholine) and PrimaFresh 606 (PF606; without morpholine), on both apple peels and on whole fruits. Significant synergistic activity between EF100 supplemented with PG and mild heat (55°C) was observed on orange skins (**Figure 7**). However, no such synergistic effects were observed in carnauba-based apple wax products with (PF360) and without (PF606) morpholines on apple peels (**Figure 9**).

Similarly, while mild heating enhanced *Listeria* reduction on wax-coated apples, we did not observe a synergistic effect of PG and mild heat against *Listeria*. PF360 and PF606 exhibited similar performance on apple surfaces, although *Listeria* exhibited rapid die-off rates in pure PF606 wax coating solution (data not shown). Therefore, PF360 was selected for subsequent studies. Increasing the heating time from 2 to 5 and 10 min with PF360 plus PG resulted in slightly greater *Listeria* log reduction on apples, whether introduced by spot inoculation (fresh contamination, **Figure 11**) or by dipping (worst-case scenario, **Figure 12**). These differences may be attributable to variations in the chemical

composition of the wax (e.g., pH, oil droplet size, surfactant types). It is also possible that differences in the surface properties of oranges and apples contributed to the observed results.

e. Inhibitory effects of olive pomace extract (OPE) and mild heat on L. innocua on fresh apples

The potential synergistic activity of heated OPE was investigated as an alternative approach for apple wax formulations. Strong antimicrobial activity was observed when OPE was heated at 55°C and applied to apple peels. The heated OPE resulted in more than 4-log reductions of *E. coli* O157:H7 (**Figure 10a**) and *L. innocua* (**Figure 10b**) on apple peels within 5 min.

As shown in **Figure 13A**, incorporation of OPE into the wax coating followed by drying at 55°C resulted in reductions of *L. innocua* on spot-inoculated apples comparable to those observed with wax treatment alone at 55°C. Both treatments resulted in higher *L. innocua* reduction than wax applied at 22°C. Similarly, for dip-inoculated apples, the addition of OPE to the wax coating followed by drying at 55°C did not result in further reductions in *L. innocua* populations compared to wax treatment alone at 55°C (**Figure 13B**).

In contrast, sequential application of OPE at 55°C followed by wax coating led to a significantly greater reduction (~3.6 log CFU) of *L. innocua* on spot-inoculated apples, compared to the ~2.1 log CFU reduction achieved with 55°C water treatment followed by wax coating (**Figure 14A**). A similar trend was observed for dip-inoculated apples, although the magnitude of reduction was smaller than that observed for spot-inoculated apples (~1.9 log CFU vs. ~3.6 log CFU reduction) (**Figure 14B**). Therefore, sequential application of OPE was selected for subsequent commercial storage studies.

f. Inhibitory effects of OPE and mild heat on resident apple microbiota

The efficacy of sequential OPE application against background aerobic bacteria and YM was further evaluated. Sequential application of OPE at 55°C followed by wax coating resulted in ~ 1.1 log CFU reduction in total aerobic plate counts (**Figure 15A**) and a ~0.5 log CFU reduction in yeast and mold (YM) counts (**Figure 15B**), compared to ~0.5 log CFU and ~0.3 log CFU reductions in total aerobic plate counts and YM counts, respectively, achieved with 55°C water treatment followed by wax coating (**Figure 15**).

Objective 4. Evaluate the influence of the optimal synergistic treatments identified in Objectives 2 and 3 on the quality (including color), microbial load (endogenous), and shelf life of fruit during storage.

a. Inhibitory effects of carnauba wax supplemented with OPE and mild heat on microbial buildup on oranges during 4 weeks of storage at 4°C

The synergistic effect of EF100 (carnauba-based wax for citrus) supplemented with OPE and mild heat (55°C) on microbial buildup in oranges was evaluated during 4 weeks of storage at 4°C. Overall, oranges treated with OPE-supplemented EF100 and mild heat exhibited slower microbial buildup compared to those treated with deionized water (control) or EF100 with mild heat (**Figure 16**). However, no difference in populations was observed from calyx after 4 weeks of storage, indicating rapid microbial buildup in the calyx region. In addition, no significant differences were found between total plate count (TPC) and YM populations, suggesting that the observed microbial buildup was primarily attributable to fungal spoilage.

b. Effects of carnauba wax supplemented with OPE and mild heat on quality changes of oranges during 8 weeks of storage at 4°C

The synergistic effect of EF100 supplemented with OPE and mild heat (55°C) on the quality change of oranges was evaluated during 8 weeks of storage at 4°C. Overall, oranges treated with OPE-supplemented wax + mild heat exhibited slower discoloration (**Figure 17a** and **b**) and weight loss (**Figure**

17c) compared to those treated with deionized water (control). However, no significant differences were observed between oranges treated with OPE-supplemented wax + mild heat and wax + mild heat, indicating that wax + mild heat treatment was also effective in reducing discoloration and weight loss of oranges during cold storage.

c. Impact of OPE and mild heat on the quality of apples during commercial storage

The impact of OPE treatment on apple quality during commercial storage was evaluated by assessing both external appearance and internal quality attributes. Gloss values remained comparable across all treatments before and after storage (**Table 1**). Similar trends were observed for fruit color (**Table 2**), indicating that OPE treatment did not affect the external appearance of the apples either immediately after treatment or after storage.

A general reduction in fruit weight, diameter, and firmness was observed after storage (data not shown), which is expected during prolonged cold storage; however, these changes were not influenced by OPE treatment. In addition, titratable acidity and total soluble solids remained stable after the 12-week storage period across all treatments. No external disorders (e.g., superficial scald, soft scald, lenticel decay) or internal disorders (e.g., water core, internal browning, or cavity formation) were observed across treatments, indicating that the application of OPE did not negatively affect apple quality during commercial storage.

During 12 weeks of commercial cold storage, total aerobic counts (**Figure 18A**) and YM counts (**Figure 18B**) on OPE-treated apples remained relatively stable. In contrast, other treatment groups exhibited a general reduction in total aerobic counts and a slight increase in YM populations over the storage period (**Figure 18**).

Outcomes and Accomplishments

- Demonstrated that plant-derived antimicrobials (olive pomace extract [OPE] and propyl gallate [PG]) combined with mild heat (40°C) can achieve synergistic inactivation of bacterial pathogens in wax formulations.
- Demonstrated that wax formulations supplemented with plant-derived antimicrobials combined with dry heat (55°C) can achieve synergistic inactivation of bacterial pathogens on citrus.
- Demonstrated that optimized treatment (55°C, 2 min) reduced microbial buildup, discoloration, and weight loss of citrus during cold storage.
- Provided commodity-specific insights, including the use of heated OPE pretreatment as a promising alternative approach for apples.
- Disseminated findings through scientific presentations to support knowledge transfer to the produce safety community.

APPENDICES

Publications and Presentations

Presentation:
Kim, Y., Cha, T., Na, S., Hahn, J., Choi, I., & **Nitin, N**. (Jul 2025). Synergistic Inactivation of Bacterial Pathogens Using Food-Grade Phenolic Derivatives with Mild Heat in Wax Coatings on Citrus. *2025 IAFP Annual Meeting*, Cleveland, OH, USA.

Budget Summary

This project was awarded $336,648 in research funds, and the majority of funds were spent to complete the project objectives.

Figures 1–18 and Tables 1–2 (see below)

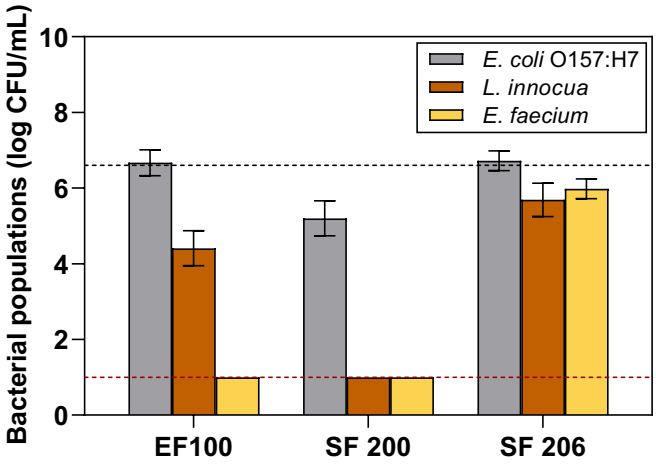

Figure 1. Inhibitory activities of the commercial wax products against bacterial pathogens (*E. coli* O157:H7, *L. innocua*, and *E. faecium*).

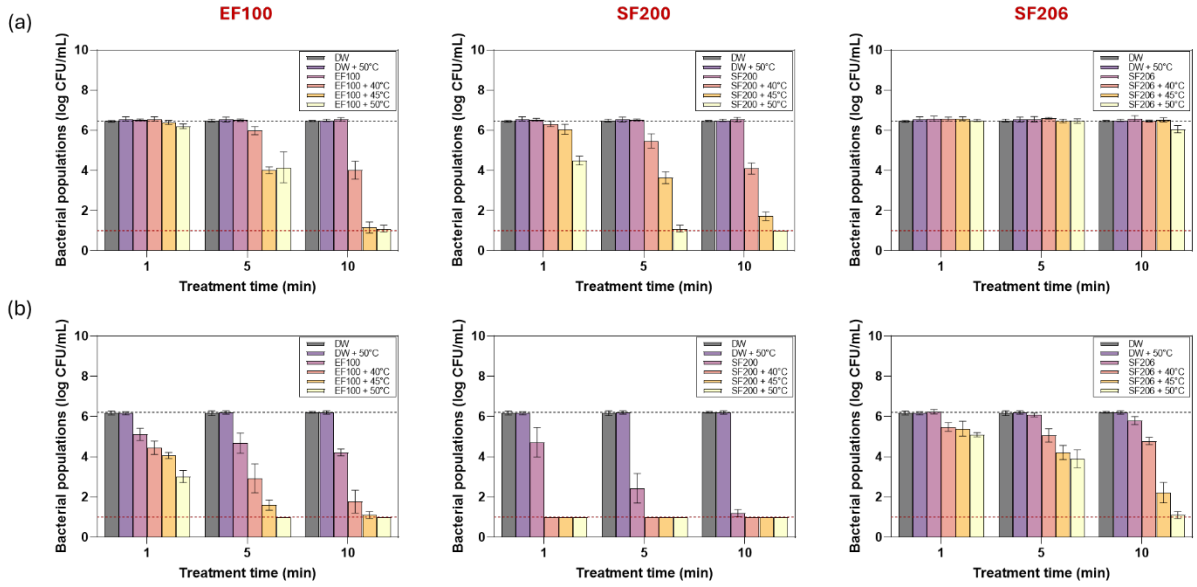

Figure 2. Inhibitory activities of commercial wax products (EF100, SF200, and SF206) against (a) *E. coli* O157:H7 and (b) *L. innocua* at room temperature (22°C), 40, 45, and 50°C.

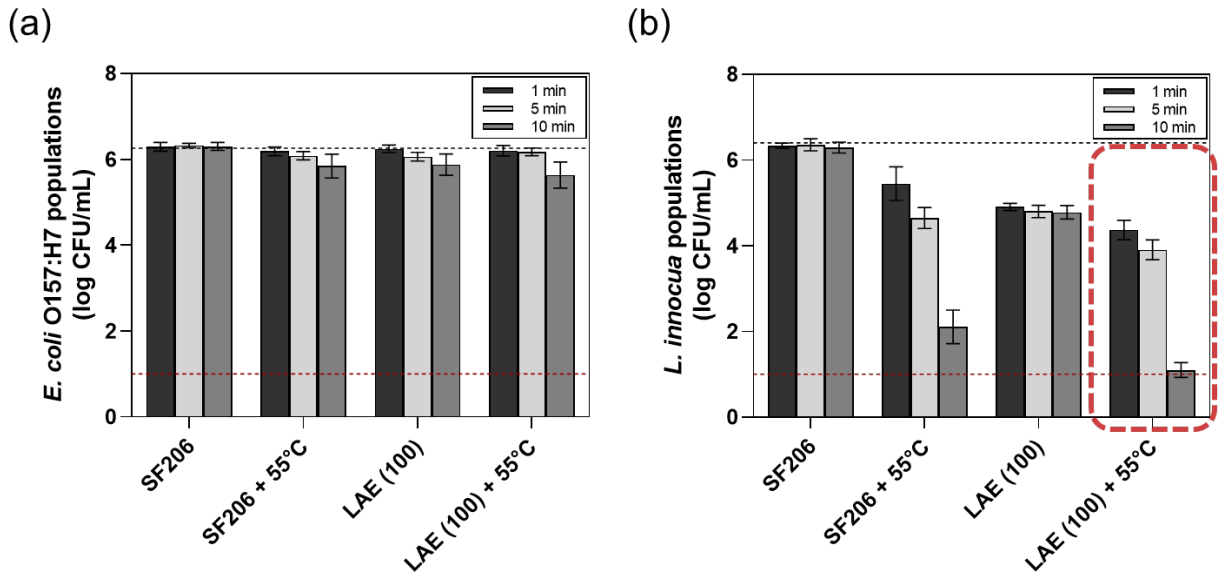

Figure 3. Synergistic antimicrobial activities of lauric arginate (LAE) with mild heat (55°C) in SF206 against (a) *E. coli* O157:H7 and (b) *L. innocua*.

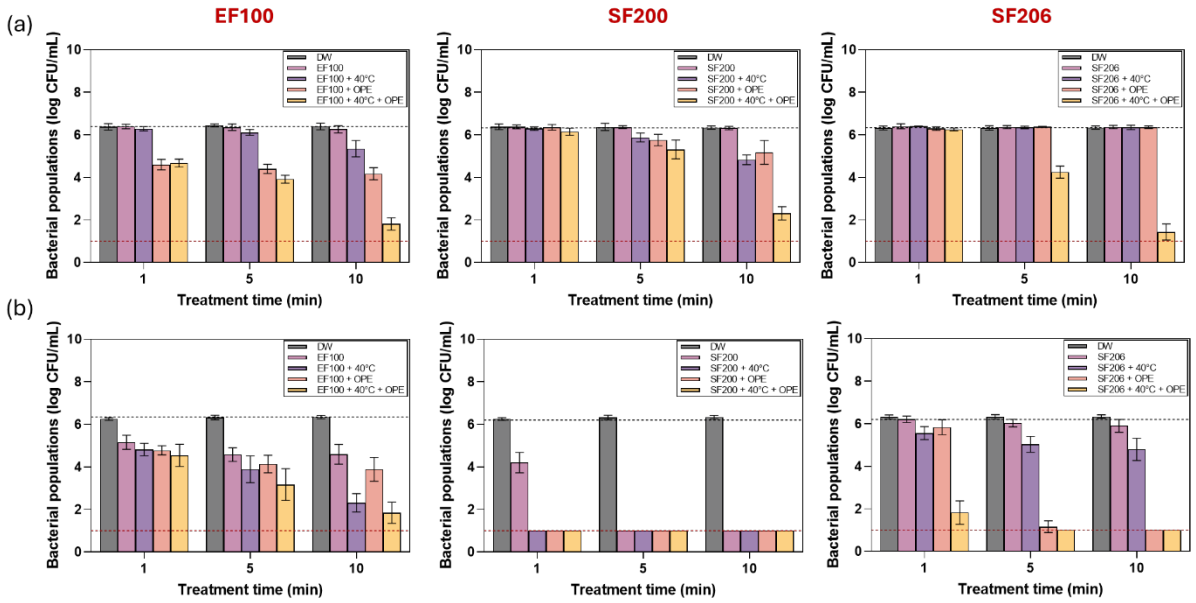

Figure 4. Synergistic antimicrobial activities of OPE and mild heat (40°C) in commercial wax products (EF100, SF200, and SF206) against (a) *E. coli* O157:H7 and (b) *L. innocua*.

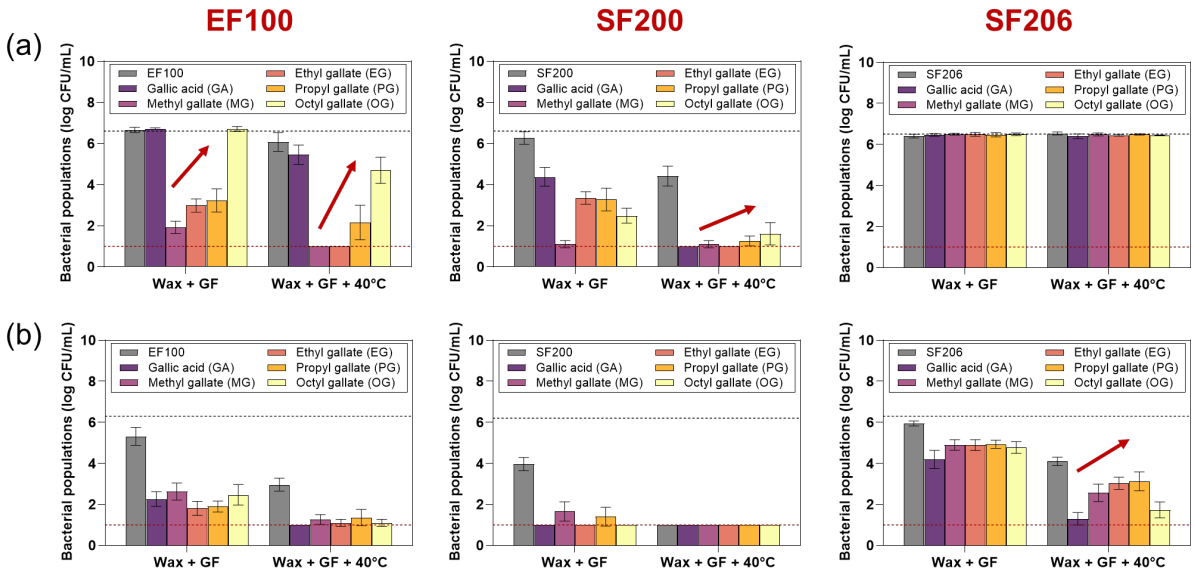

Figure 5. Synergistic antimicrobial activities of gallate family (GF) compounds with mild heat (40°C) in commercial wax products (EF100, SF200, and SF206) against (a) *E. coli* O157:H7 and (b) *L. innocua*.

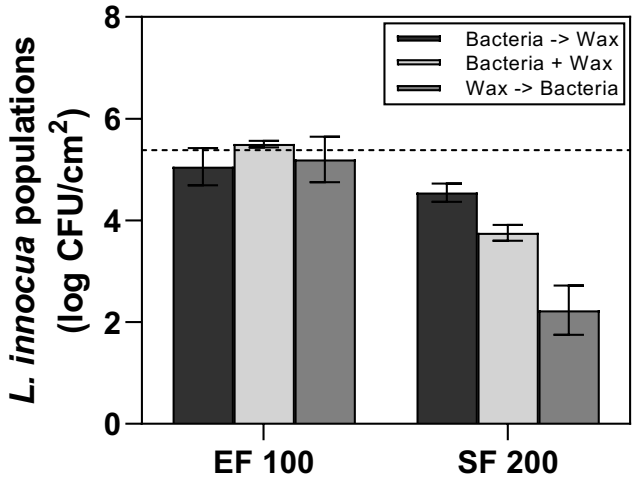

Figure 6. Inhibitory activities of the commercial wax on orange skin. The inhibitory activities of the carnauba-based wax coating (EF100 and SF200) were tested on orange skins using three different approaches. *L. innocua* cells were spread-inoculated onto orange skin before applying the wax coating (10 µL/cm^2) (Bacteria → Wax), co-inoculated with wax formulations on orange skin (Bacteria + Wax), or inoculated after applying the wax coating on orange skin (Wax → Bacteria).

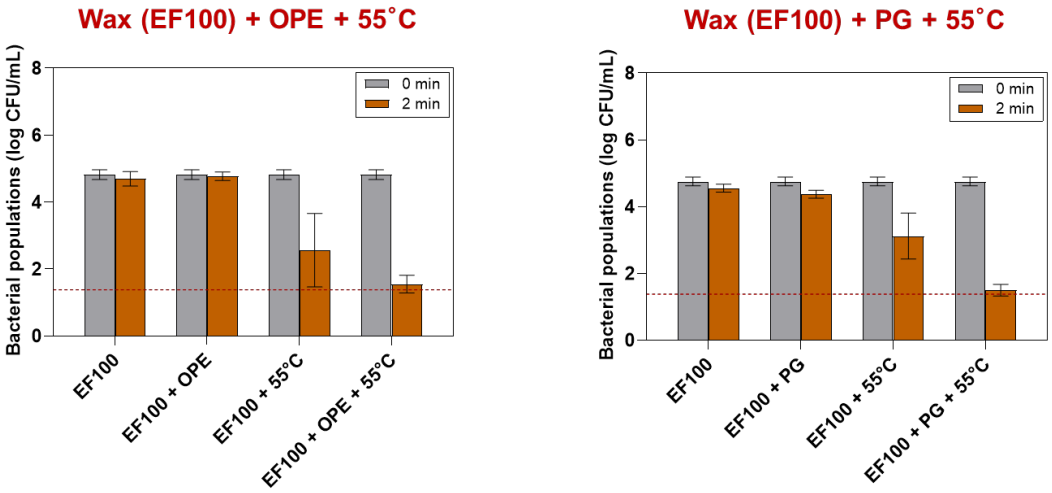

Figure 7. Synergistic antimicrobial activities of selected antimicrobial compounds (OPE [1.0 mg GAE/mL] or PG [5 mM]) and dry heat (55°C) in EF100 against *E. coli* O157:H7 on orange skin.

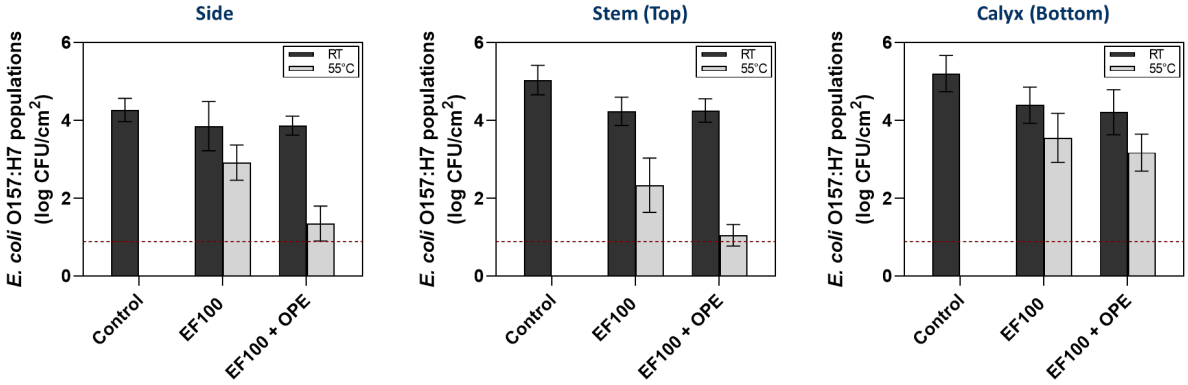

Figure 8. Synergistic antimicrobial activity of OPE and dry heat against *E. coli* O157:H7 on whole oranges. *E. coli* O157:H7 cells were pre-inoculated on the side (left), stem (middle), and calyx (right) of whole orange and treated with EF100 supplemented with OPE and dry heat (55°C) for 5 min.

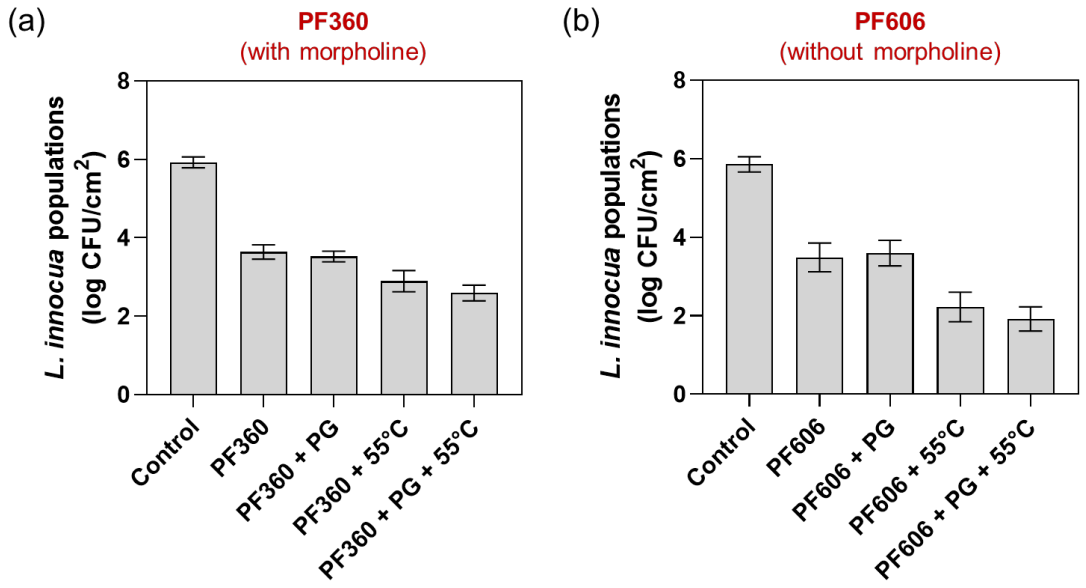

Figure 9. Limited synergistic activity between propyl gallate (PG) and mild heat (55°C) against *L. innocua* in apple wax products: (a) PF360 (carnauba-based wax with morpholine) and (b) PF660 (carnauba-based wax without morpholine).

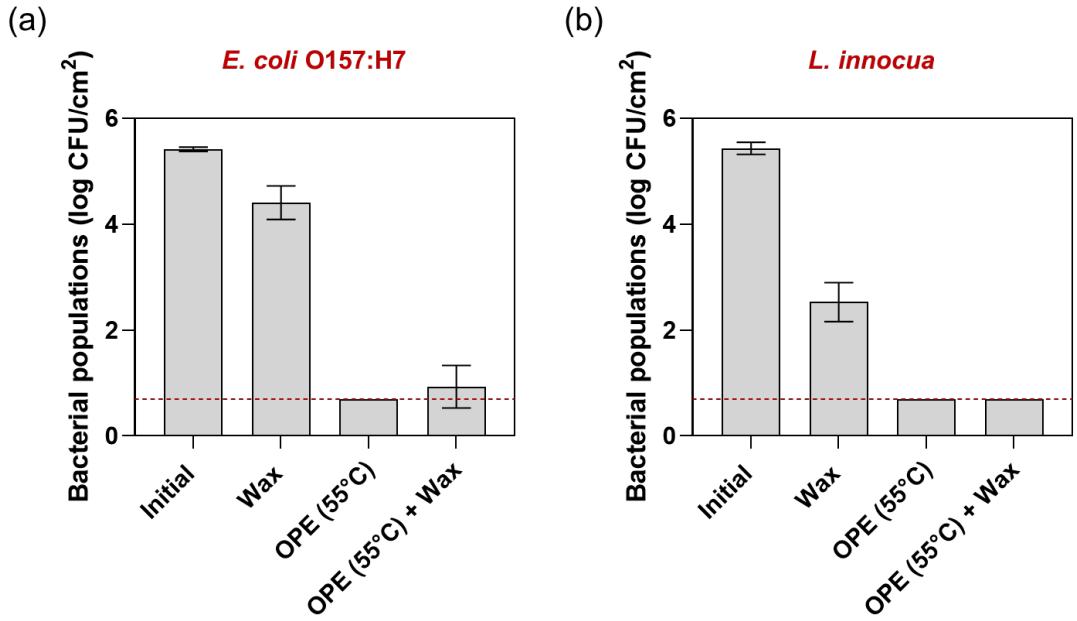

Figure 10. Antimicrobial activity of heated OPE (55°C) on apple peels against (a) *E. coli* O157:H7 and (b) *L. innocua*.

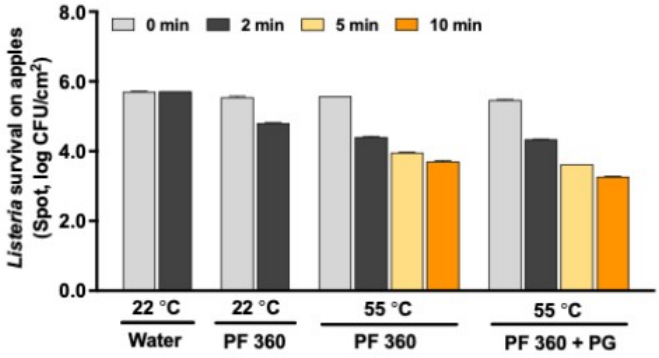

Figure 11. Effect of PF360, with or without propyl gallate (PG), combined with mild heat (55 °C), on *Listeria innocua* spot-inoculated on apples across different heating durations.

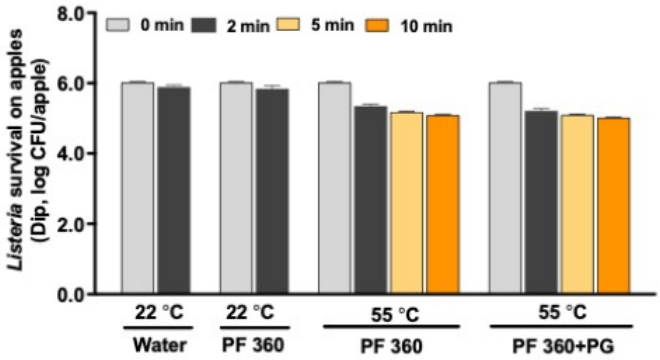

Figure 12. Effect of PF360, with or without propyl gallate (PG), combined with mild heat (55 °C), on *Listeria innocua* dip-inoculated on apples at different heating durations.

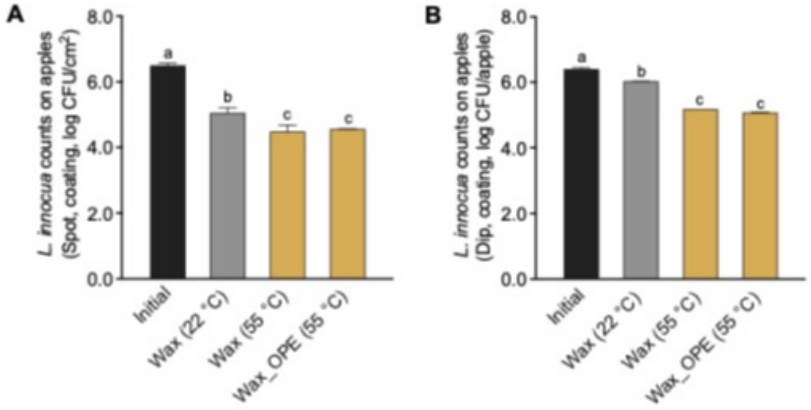

Figure 13. Survival of *Listeria innocua* spot-inoculated (A) and dip-inoculated (B) on Gala apples coated with wax with or without OPE and dried at 55°C for 5 min. Different letters (a–d) above the bars indicate significant differences among treatments ($P < 0.05$). Data are presented as mean ± SEM (n = 15).

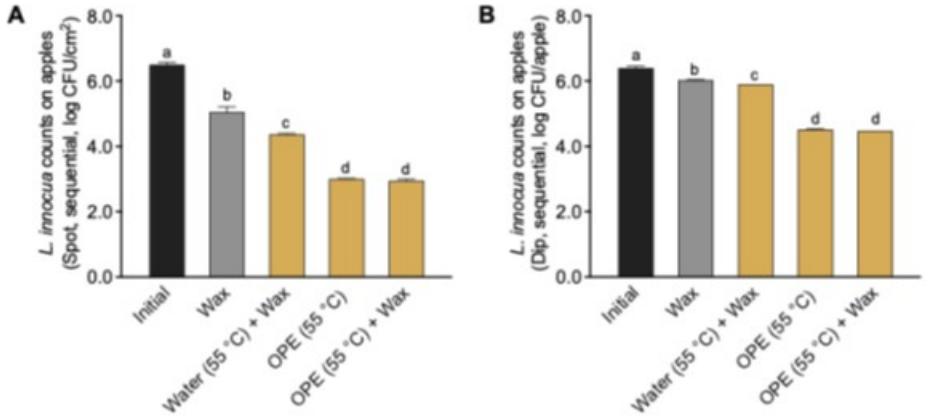

Figure 14. Survival of *Listeria innocua* spot-inoculated (A) and dip-inoculated (B) on Gala apples treated with OPE solution at 55°C for 5 min followed by wax coating. Different letters (a–d) above the bars indicate significant differences among treatments ($P < 0.05$). Data are presented as mean ± SEM (n = 15).

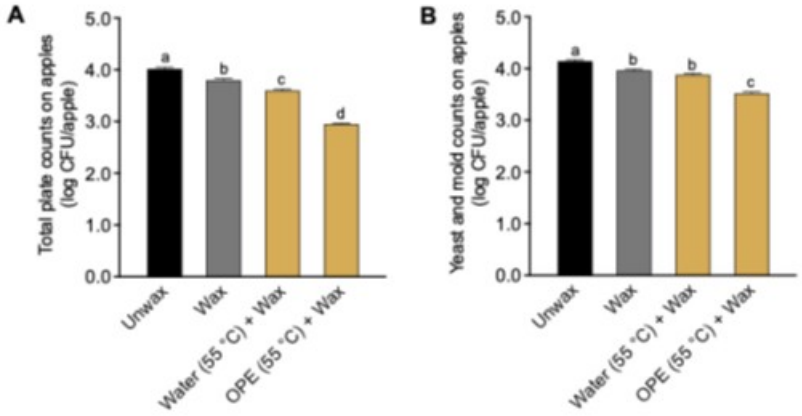

Figure 15. Resident microbiota of Gala apples treated with OPE solution at 55°C for 5 min followed by wax coating. (A) Total aerobic plate counts; (B) Yeast and mold counts. Different letters (a–d) above the bars indicate significant differences among treatments ($P < 0.05$). Data are presented as mean ± SEM (n = 40).

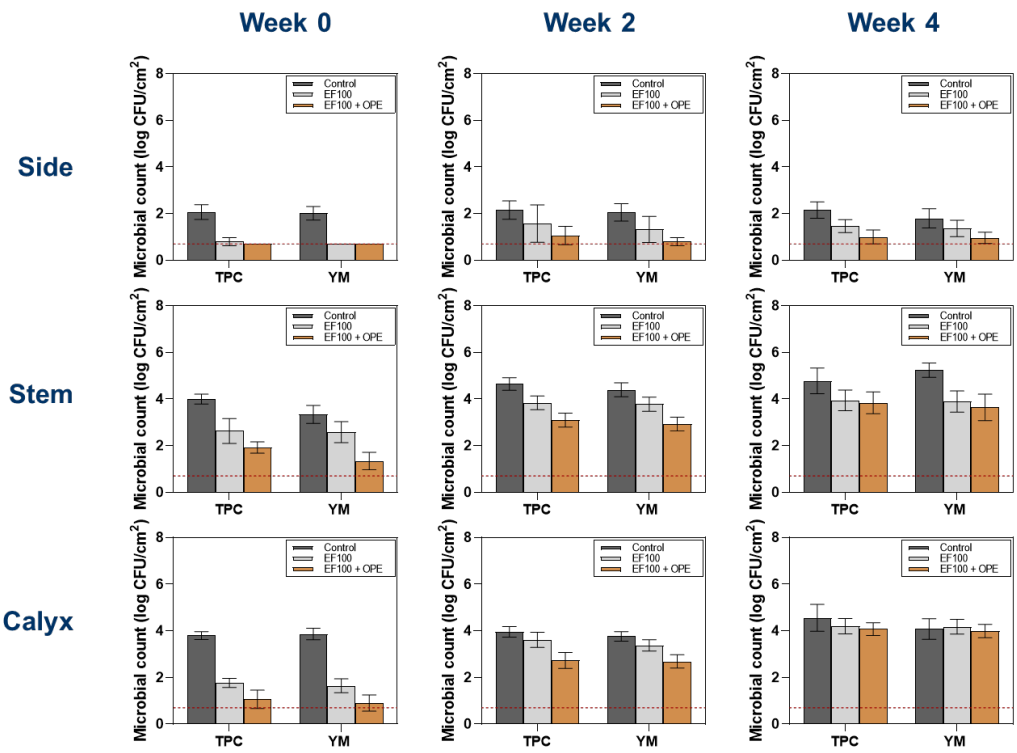

Figure 16. Microbial buildup (total plate count [TPC] and yeasts & molds [YM]) on oranges treated with deionized water (DW), EF100 + mild heat, and OPE-supplemented EF100 + mild heat during 4 weeks of storage at 4°C.

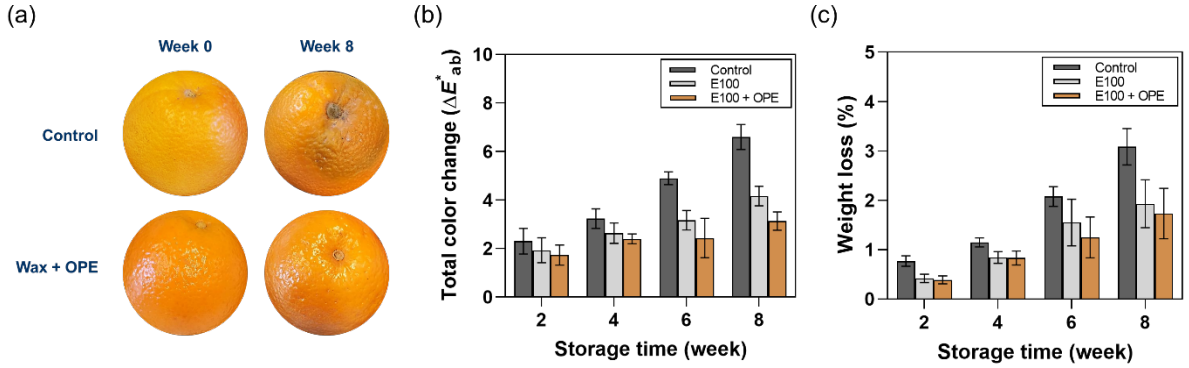

Figure 17. Quality changes of oranges treated with OPE-supplemented EF100 + mild heat during 8 weeks of storage at 4°C: (a) optical images, (b) total color change, and (c) total weight loss of oranges treated with deionized water (DW), EF100 + mild heat, and OPE-supplemented EF100 + mild heat.

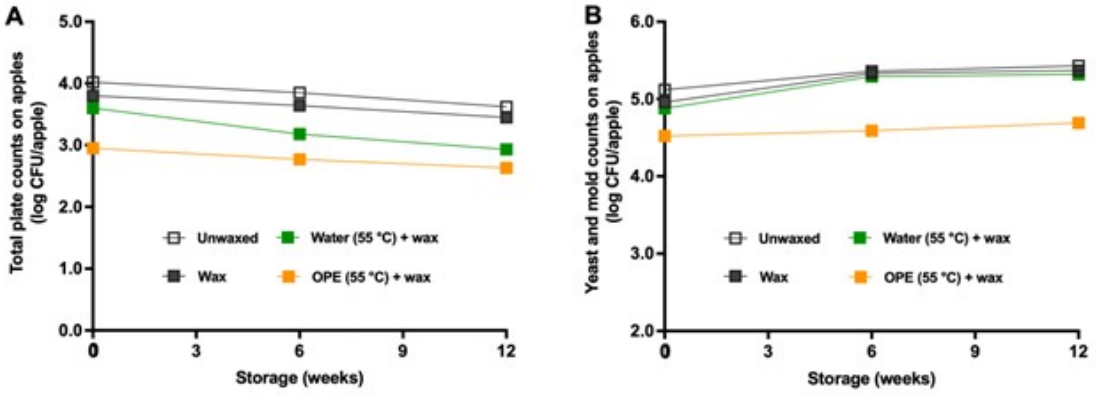

Figure 18. Resident microbial counts on Gala apples sequentially treated with OPE solution or water at 55°C for 5 min, followed by wax coating, during 12 weeks of commercial cold storage. (A) Total aerobic plate counts. (B) Yeast and mold counts. Mean ± SEM (n = 40).

Table 1. Gloss index (GU) of Gala apples before and after commercial cold storage

	Blushed		Shaded	
	0-week	12-week	0-week	12-week
Untreated	3.8±0.2	3.2±0.1	3.8±0.2	3.1±0.1
Wax only	11.7±0.3	11.4±0.2	10.9±0.2	11.0±0.2
Water (55 °C) + wax	11.6±0.3	11.1±0.2	11.3±0.3	11.1±0.2
OPE (55 °C) + wax	12.3±0.3	11.8±0.3	11.4±0.3	11.3±0.2

Wax only: PrimaFresh 360. Data are reported as mean ± SEM (n = 10), with 10 measurements per apple per side.

Table 2. Color measurements of Gala apples before and after commercial cold storage

	L*		a*		b*	
	0-week	12-week	0-week	12-week	0-week	12-week
	Blushed					
Untreated	51.99±0.40	51.95±0.51	27.08±2.08	25.13±0.34	11.28±0.24	13.25±0.40
Wax only	54.27±0.35	50.58±0.47	23.58±0.28	27.08±0.37	11.67±0.25	13.64±0.34
Water (55 °C) + wax	51.02±0.28	48.34±0.48	26.03±0.25	26.72±0.26	11.71±0.20	12.20±0.23
OPE (55 °C) + wax	52.31±0.43	49.99±0.33	25.45±0.37	25.84±0.30	11.72±0.27	12.84±0.29
	Shaded					
Untreated	63.37±0.70	67.29±6.56	19.87±0.74	20.64±0.72	17.78±0.45	19.14±0.71
Wax only	67.39±0.70	58.69±0.70	15.18±0.78	24.05±0.56	18.59±0.52	18.19±0.61
Water (55 °C) + wax	63.71±0.62	61.25±0.63	21.40±0.68	22.97±0.61	17.81±0.54	20.51±0.46
OPE (55 °C) + wax	62.85±0.78	65.07±0.60	21.75±0.76	20.28±0.61	17.12±0.43	21.22±0.38

Wax only: PrimaFresh 360. L* represents lightness; a* represents the green-to-red axis (positive values indicate red); and b* represents the blue-to-yellow axis (positive values indicate yellow). Data are reported as mean ± SEM (n = 10), with 10 measurements per apple per side.

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