CPS 2022 RFP – FINAL PROJECT REPORT

Project Title:
Supplementing food antimicrobials in commercial edible coatings to enhance the safety and extend the shelf-life of stone fruits

Project Period:
January 1, 2023 – December 31, 2024

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Objectives:

1. Characterize the physical, mechanical, and antimicrobial properties of films casted from commercial stone-fruit coatings supplemented with food antimicrobials.
2. Evaluate the reduction of inoculated pathogens, native yeasts, and molds/fungi, and evaluate the quality of stone fruits after coating and during storage.

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FINAL REPORT

Summary of Findings and Recommendations

Commercial food preservatives of lauric arginate (LAE), sorbic acid (SA), sorbate, benzoate, benzoic acid, propionic acid, and parabens were generally more active at lower pH between 4 and 7 against *Salmonella* Enteritidis H4267 and *Listeria monocytogenes* Scott A. LAE was the most active and had synergistic activity when combined with sorbic acid or parabens at pH 6.0. The commercial stone fruit coatings had alkaline pH, negative charges, and high fat content. When adjusting to pH 6.0 and dissolving with 1% antimicrobial, only the EXC 7037 coating with original pH of 8.7 remained visually stable and was chosen. The combination of LAE and organic acids had stronger activity against *Salmonella* Enteritidis H4267 and *L. monocytogenes* Scott A in the EXC 7037 coating after adjusting pH to 6.0, and the 200 ppm LAE and 5,000 ppm SA combination was the most active, achieving 3.42 log CFU/mL reduction against *Salmonella* after 90 min at room temperature but only about 1 log CFU/mL or less reduction against *L. monocytogenes*. Parabens were active in the EXC 7037 coating without pH adjustment, reducing both pathogens by more than 8 log CFU/mL in 90 min at room temperature. With these findings, four formulations based on the EXC 7037 coating were chosen to coat fresh peaches: (A) 0.05% LAE and 0.5% SA at pH 6.0, (B) 0.1% LAE and 1.0% SA at pH 6.0, (C) 1.0% parabens at pH 8.7, and (D) 2.0% parabens at pH 8.7.

Fresh peaches were brushed, washed with deionized water, dried, and spot-inoculated with *Salmonella* or *L. monocytogenes* cocktail composed of equal populations of five strains. Separate peaches without inoculation were used to evaluate native fungi and quality. Each peach was sprayed with 1 mL of the chosen coating formulation, with controls being that sprayed with the coating without antimicrobial and that without coating. The survival of microorganisms and the quality of peaches were evaluated every 5 days during 20-day storage at 0°C and 85% relative humidity. Six peaches from each of the two different harvest seasons were used, giving 12 replicates for each data point. Peaches sprayed with formulations (A) and (B) were stored in open aluminum trays to simulate transportation in large containers, while those with formulations (C) and (D) were placed in perforated plastic pouches before placing in aluminum trays to simulate packing in boxes. When compared to the coating control without antimicrobials, formulations (A) and (B) increased *Salmonella* reduction by up to 1.64 log CFU/fruit (on day 20), while the impact on *L. monocytogenes* was insignificant; formulations (C) and (D) increased the reduction of *Salmonella* and *L. monocytogenes* by up to 1.81 and 0.44 log CFU/fruit (on day 20), respectively. Another strategy was developed by first spraying 1% or 2% parabens on peaches followed by applying the EXC 7037 coating without pH adjustment, resulting in similar pathogen populations during storage – an "instant kill" effect; the reduction by 1% and 2% parabens was increased by up to 2.19 and 2.55 log CFU/fruit for *Salmonella* and 0.72 and 0.87 log CFU/fruit for *L. monocytogenes* (on day 20), respectively. Supplementing the coating with the antimicrobials did not impact the coating to inhibit native fungi and preserve peach quality evaluated for total soluble solids content, titratable acidity, pH, weight loss, and color (L, a*, b*).

At the studied conditions, supplementing antimicrobials in the coating was more effective in inhibiting *Salmonella* than *L. monocytogenes* without altering the current coating practice used in the packing house. To further reduce pathogens on stone fruits, the produce industry may want to explore coatings feasible at acidic conditions to utilize the activity of the antimicrobials, especially those at pH 4 and below to inhibit the growth of foodborne pathogens using acidity. Alternatively, the packing house may install an additional sprayer to apply antimicrobials such as parabens to provide an instant kill of pathogens and native fungi, followed by spraying the stone fruit coating to minimize the impact of coating composition and achieve sufficient inhibition of pathogens.

Abstract

Coatings (waxes) and cold storage improve the quality and shelf-life of stone fruits, but current commercial stone fruit coatings may not inhibit incident foodborne pathogens. The main project objective was to evaluate the pathogen reduction and quality of peaches after applying commercial stone fruit coatings supplemented with commercial food antimicrobials. Lauric arginate (LAE) had synergistic antimicrobial activity with sorbic acid (SA) or parabens at pH 6.0. The alkaline stone fruit coating was then adjusted to pH 6.0 before supplementing LAE and SA. Fresh peaches were brushed, washed, and spot-inoculated with *Salmonella* or *Listeria monocytogenes* cocktails, followed by spraying with the coating supplemented with LAE (0.05% and 0.1%) and SA (0.5% and 1.0%). The residual pathogen was enumerated every 5 days during storage at 0°C and 85% relative humidity for 20 days. Peaches coated without antimicrobials and uncoated peaches were studied as controls. When compared to the coating control without antimicrobials, LAE and SA in the coating increased *Salmonella* reduction by up to 1.64 log CFU/fruit, while the impact on *L. monocytogenes* was insignificant. When parabens were directly dissolved at 1% or 2% in the alkaline coating, the reduction of *Salmonella* and *L. monocytogenes* increased by up to 1.81 and 0.44 log CFU/fruit, respectively. Another strategy was developed by spraying 1% or 2% parabens on peaches followed by applying the alkaline coating, resulting in the respective reduction increase by up to 2.19 and 2.55 log CFU/fruit for *Salmonella* and 0.72 and 0.87 log CFU/fruit for *L. monocytogenes*. Supplementing with the antimicrobials did not have negative impacts on preserving peach quality in terms of native fungi inhibition, weight loss, color, pH, titratable acidity, and total soluble solids. These results suggest the potential of adopting antimicrobials to coat peaches, but future studies are needed to further increase pathogen reduction, especially *L. monocytogenes*.

Background

Peach (*Prunus persica*) is a stone fruit that is generally considered low risk, but this commodity has been recently associated with outbreaks involving *Listeria monocytogenes* and *Salmonella*. According to the U.S. Centers for Disease Control and Prevention, multi-state foodborne illness outbreaks were reported after consumption of peaches contaminated with *L. monocytogenes* [1] and *Salmonella* Enteritidis.[2] Peaches may encounter foodborne pathogens at different stages of the supply chain. The stone fruit industry uses two main strategies to keep the quality of the peaches, namely, storing the fruits in cold conditions (0-5°C and 80-95% relative humidity) and coating the fruits with food grade materials. Commercial coatings are typically applied to fruits in liquid form by dipping, spraying, brushing, or dripping. Edible coatings form a semi-permeable membrane on the surface of the fruits, acting as a barrier against gases, moisture, and solute movements. Overall, the use of edible antimicrobial coatings to inhibit foodborne pathogens in whole peaches is relatively scarce. Therefore, the objective of this study was to evaluate the reduction of pathogenic bacteria and native fungi as well as the impact on the quality of fresh peaches sprayed with a commercial stone fruit coating supplemented with different antimicrobials during storage.

Research Methods and Results

Methods

Lauric arginate (LAE) was kindly provided by Vedeqsa Inc. (New York, NY), commercially available as Mirenat-TT. Methyl- and propyl parabens were products of Thermo Fisher Scientific (Waltham, MA) and were used in combination at 2:1 mass ratio. A stock solution with 10% parabens in 70% v/v ethanol was prepared for mixing with the coating or diluting with deionized water for other uses. Organic acids and their salts (>99% purity) were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Broth and agar media

were obtained from Thermo Fisher Scientific Inc. (Pittsburgh, PA). Fresh and unwashed peaches (*Prunus persica* L. Batsch) were purchased from Safe Fresh Fruit (Fresno, CA) and were stored in a disinfected cold room at 0°C upon receipt. Selection of the peaches was based on their similar size, color, and lack of physiological defects. The selected peaches were brushed and washed with deionized water and then kept on aluminum trays to allow drying of excess water on the peach surface. The coatings (EXC 7037, EXC 7036, EXC 6057, and EXC 6095) were provided by Pace International, LLC (Wapato, WA). All the coatings except EXC 7036 are stone fruit coatings, and their compositions are trade secret.

For *in vitro* assays, *L. monocytogenes* Scott A and *Salmonella* Enteritidis ATCC H4267 were tested. For experiments with peaches, separate cocktails of *Salmonella* and *L. monocytogenes* were used. The *Salmonella* cocktail included five strains: *Salmonella* Enteritidis (ATCC H4267 and S5-371), *Salmonella* Javiana (S5-406), and *Salmonella* Typhimurium (A4-737, and S5-370). Five strains were also used for the *L. monocytogenes* cocktail, including Scott A, Mack (X1-0001, and F6-0367), and 4B (R2-0574, and F2-501). The strains were obtained from the culture collection of the Department of Food Science at the University of Tennessee in Knoxville. All strains were kept at −80°C in tryptic soy broth (TSB) with 20% v/v glycerol. The population of the cocktails was about 9 log CFU/mL and was confirmed by spread-plating 0.1 mL culture on modified Oxford agar (MOX, Difco, Becton, Dickson and Company, Sparks, MD) for *L. monocytogenes* and xylose lysine deoxycholate agar (XLD, Difco, Becton, Dickson and Company) for *Salmonella*, followed by incubation at 32 and 37°C for 24 h, respectively, before enumeration.

The efficacy of the antimicrobials in TSB adjusted to pH 4.0-7.0 against one individual strain of *Salmonella* and *L. monocytogenes* was determined using the microbroth dilution method.[3] The minimum inhibitory concentration (MIC) was set as the lowest concentration of the antimicrobial that inhibited bacterial growth corresponding to an optical density change of <0.05 at 630 nm (ΔOD_{630nm}). To determine the minimum bactericidal concentration (MBC), 0.1 mL aliquots from each microtiter well showing no- or minimal growth was spread-plated on tryptic soy agar, followed by incubation at 32 or 37°C for 48 h; the MBC was defined as the antimicrobial concentration giving ≥3 log decrease in viable cells in comparison to the initial inoculum. Based on the individual MICs, the antimicrobials were also tested when combined. The checkerboard method was used to determine the fractional inhibitory concentration index (FICI) of the antimicrobial combinations (Eq. 1).[4] The results were interpreted as synergy (FICI ≤0.5), partial synergy (0.5 <FICI ≤0.75), addition (0.75 <FICI <1.0), indifference (1.0 <FICI ≤4), or antagonism (FICI >4.0).[5] Antimicrobials were chosen based on these *in vitro* results.

$$FICI = \frac{MIC\ of\ antimicrobial\ A\ in\ combination}{MIC\ of\ antimicrobial\ A\ alone} + \frac{MIC\ of\ antimicrobial\ B\ in\ combination}{MIC\ of\ antimicrobial\ B\ alone} \quad (1)$$

The approximate composition and physical properties of the coatings were evaluated. Pathogen survival in the EXC 7037 coating supplemented with antimicrobials was evaluated. The EXC 7037 coating was then dissolved with antimicrobials before applying on peaches inoculated with a pathogen cocktail, and the survival of pathogens during storage at 0°C and 85% relative humidity was determined every 5 days. Another set of experiments was studied during room temperature (RT, 21°C) storage for 5 days. Peaches without pathogen inoculation were used to evaluate the survival of native fungi, as well as the quality. Uncoated peaches and peaches applied with the coating without antimicrobials were evaluated as controls. For this set of experiments, fresh peaches were spot-inoculated with the bacterial cocktail by placing 200 µL of the inoculum (~7 log CFU/mL) at 10 different spots (20 µL each) around the stem. Inoculated peaches were kept at RT in a biosafety cabinet for 4 h to allow the inoculum to dry. The inoculated peaches were each sprayed with the commercial coating using a gravity-feed dual-action air-nozzle sprayer (PointZero Model No. Elite-125X, Tamarac, FL). Each peach was sprayed with three pulls above the stem (about 1 mL) at RT at a distance that ensured uniform application on the fruit surface.

Four hours after drying, the peaches were placed on unsealed sanitized aluminum trays (6 peaches/tray), which simulates transportation in large containers,[6] and moved to the storage room. Another set of peaches applied with parabens were packed in perforated plastic pouches before placing in aluminum trays, which simulates packing in boxes. Peaches (n = 6) were sampled on day 0, 5, 10, 15, and 20 for the enumeration of bacteria. Two separate batches of peaches from two different harvesting seasons were used, giving the total number of replicates to 12 for each data point. Uninoculated peaches were treated and stored in the same conditions to evaluate the survival of native fungi and physicochemical quality attributes. All results were compared with one way analysis of variance followed by Tukey multiple comparisons at a significance level of 0.05.

Results

The results are presented in the Appendix. **Tables 1** and **2**, respectively, show the MICs and MBCs of the antimicrobials against *L. monocytogenes* and at *Salmonella* different pH. According to the results, LAE was identified as the strongest antimicrobial and therefore was tested for the binary combinations with sorbic acid, parabens, or sodium benzoate. These antimicrobials had strong activity against both Gram-negative and Gram-positive bacteria, generally stronger in more acidic conditions. When evaluated in combinations based on the FICI, different modes of interactions were observed (**Table 3**). According to the results, pH 6.0 was identified as the optimal acidity for the combination of LAE with parabens or sorbic acid.

The composition of the coatings is shown in **Table 4**. The zeta potential (surface charge property) of the commercial coatings is shown in **Fig. 1**. The zeta-potential and droplet size distribution of the EXC 7037 coating supplemented with antimicrobials (1%) at different pH are shown in **Figs. 2** and **3**, respectively. When the four commercial coatings were dried at RT and increased temperatures, no viable films formed (**Fig. 4**). The visual solubility of the antimicrobials in the commercial coatings as well as the visual stability of coatings after adjusting to pH 4.0-6.0 was evaluated, shown in **Table 5** for samples at pH 6.0. All the coatings had alkaline pH ranging from 8.7 to 10.6, and only the coating coded with EXC 7037 (pH 8.7) remained stable after adjusting pH to 6.0 and the addition of antimicrobials but was unstable at lower pH, while the other three coatings showed visual separation even at pH 6.0. The EXC 7037 coating was then used in later studies.

The antibacterial activity of the chosen antimicrobial combinations in the EXC 7037 coating before and after adjusting pH from 8.7 to 6.0 was characterized. Log reduction kinetics of *L. monocytogenes* and *Salmonella* Enteritidis in the coating was determined after incubation at RT for 1, 30, 60, and 90 min. The 90 min results are presented in **Table 6**. Adjusting pH to 6.0 greatly enhanced the antimicrobial activity that was much stronger for *Salmonella* Enteritidis than *L. monocytogenes*. For antimicrobials combined with LAE, the most effective reduction was 3.4 log CFU/mL of *Salmonella* Enteritidis by 200 ppm LAE and 5,000 ppm sorbic acid at pH 6.0. While for parabens, a higher antimicrobial concentration led to a greater reduction, and the 2% w/v (20,000 ppm) parabens reduced both pathogens to be below the detection limit after 90 min. The alkaline pH, high fat content, negative charges, and complex composition of the commercial coatings are possible causes that make the antimicrobials much less effective than in the microbial growth media.

With the *in vitro* results, the experiments were conducted with two separate batches of peaches by evaluating the reduction of pathogens and native fungi by LAE and sorbic acid combination during storage in open trays. The populations of pathogens and native fungi in the peaches during storage at 0°C are shown in **Fig. 5**, and the log reduction and native fungi population at the end of storage at 21°C or 0°C are shown in **Tables 7** and **8**, respectively. When compared to the coating treatment without antimicrobials, LAE and sorbic acid inhibited the *Salmonella* cocktail in the peaches but was ineffective against the *L. monocytogenes* cocktail. The uncoated peaches showed the highest reductions of the pathogens, likely

due to dehydration because the peaches were not covered. For native fungi, LAE and sorbic acid showed significant inhibitions when compared to the coating control without antimicrobials ($p < 0.05$). Additionally, as part of the quality evaluation, the total soluble solids (TSS), titratable acidity (TA), pH, weight loss (%), and color (L, a*, b*) of the uninoculated peaches during storage were determined (**Tables 9-12**). Overall, all treatments had similar TA and pH during storage ($p > 0.05$), and the uncoated peaches had a similar TSS as the coated peaches during storage at 0°C ($p > 0.05$) before showing a significant higher TSS at the end of storage ($p < 0.05$). The weight loss increased during storage ($p < 0.05$) and was significantly less ($p < 0.05$) for the coated peaches than the uncoated peaches, and the addition of antimicrobials had an insignificant impact on the weight loss ($p > 0.05$). Lastly, there was no significant difference in color parameters during storage at either temperature (**Tables 11** and **12**). Our industry advisory council suggested proper packaging of the peaches should help to reduce the weight loss.

As shown in **Table 6**, the methylparaben and propylparaben (2:1, w:w) mixture also had strong antimicrobial activities in the EXC 7037 coating without pH adjustment. The EXC 7037 coating supplemented with parabens directly without pH adjustment was thus tested. Two concentrations of parabens (1% and 2%) were dissolved in the EXC 7037 coating to treat peaches. The survival of pathogens and native fungi during storage at 0°C and 80-90% relative humidity for up to 20 days is presented in **Fig. 6**, with **Table 13** summarizing the reduction of pathogens and population of native fungi after 20-day storage. For the coating with 2% parabens, the reduction of *Salmonella* was 3.05±0.32 log CFU/fruit and was the highest among treatments ($p < 0.05$) on day 20, whereas only a 1.95±0.19 log CFU/fruit reduction was observed for *L. monocytogenes.* The respective reduction for the coating supplemented with 1% parabens on day 20 was 1.74±0.22 and 1.92±0.17 log CFU/fruit for *Salmonella* and *L. monocytogenes*, respectively. The reduction of *Salmonella* was significantly higher than the uncoated treatment ($p < 0.05$), but the opposite was true for *L. monocytogenes*. There was no significant difference in the native fungi among the treatments ($p > 0.05$) after storage (Table 13). The quality of the coated and uncoated peaches was evaluated by determining the color and weight loss (%). In both coated and uncoated peaches, the weight loss increased during storage but was less than 6%, and the addition of parabens had an insignificant impact ($p > 0.05$) on the weight loss and color parameters during storage (**Table 14**).

To explore further, another scenario was tested by first spraying the paraben solution on peaches, followed by spraying the EXC 7037 coating. The bacterial population during 20-day storage at 0°C is shown in **Fig. 7**. The bacterial populations after spraying parabens were relatively constant during storage, implying an "instant kill" step. The log reductions and native fungi population at the end of storage are shown in **Table 15**. More than 3 log CFU/fruit reductions were achieved either with 2% or 1% of parabens against *Salmonella* at the end of storage, while it was around 2 log CFU/fruit reduction against *L. monocytogenes*. Different from the previous results, peaches coated only with the EXC 7037 coating (without parabens) and the uncoated peaches had higher fungi populations on day 20 than peaches coated with parabens ($p < 0.05$; Table 15). Finally, no significant difference ($p > 0.05$) in color parameters during storage was observed among treatments, while the weight loss was less than previous treatments where peaches were sprayed with coating dissolved with parabens (**Table 16** vs. **Table 14**).

Outcomes and Accomplishments

Physical properties of the commercial stone fruit coatings as impacted by supplementing antimicrobials were evaluated, and commercial food preservatives as candidates for incorporation in the coatings were identified. The reduction of pathogens inoculated on peaches and the reduction of native fungi after applying coatings with different antimicrobials were assessed, as well as the impact on peach quality. The findings may be adopted by the produce industry to supplement antimicrobials in the stone fruit coating

to inhibit pathogens, especially *Salmonella*. An alternative approach of spraying parabens on peaches before applying the stone fruit coating was additionally developed. The project findings also provide useful information for the product industry. While supplementing the studied antimicrobials in the coating is effective in inhibiting *Salmonella*, more work is needed to reduce *L. monocytogenes*. With pH and the coating composition being identified as crucial factors impacting the activity of the antimicrobials, the industry may want to explore coatings feasible at acidic conditions to utilize the antimicrobial activity, especially those at pH 4 and below to inhibit the growth of foodborne pathogens using acidity. To further increase the reduction of pathogens, the packing house may install an additional sprayer to apply antimicrobials such as parabens to provide an instant kill of pathogens and native fungi, followed by spraying the stone fruit coating to minimize the impact of coating composition and achieve sufficient inhibition of pathogens.

References

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APPENDIX

Publications and Presentations
N/A

Budget Summary
This project was awarded $324,925 in research funds. We have adjusted the budget a few times and received approval from CPS throughout the project. As of January 2025, we had about $12,000 supplies budget and $4,796 travel budget remaining. We have communicated with CPS and plan to use the supplies budget for travel to the CPS symposium and possible publication charges. Overall, we had sufficient budget to complete the original plan with modifications suggested by the industry advisory council. However, because peaches are not available throughout the year, we wish we could have had more time and budget to try additional ideas to achieve better reduction of *L. monocytogenes* on peaches.

Tables and Figures

Table 1. MIC and MBC (ppm) of antimicrobials against *L. monocytogenes* Scott A at pH 5-7.

pH	Sorbic acid		Benzoic acid		Sodium benzoate		Propionic acid		Sodium propionate		Parabens		Lauric arginate	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
5.0	100	400	200	800	300	800	200	1560	1250	10000	1560	3125	45	>50
6.0	400	3000	3125	12500	3000	5000	6000	16000	3000	>20000	3125	6250	25	50
7.0	1500	4000	12500	30000	9000	10000	8000	>50000	10000	>20000	6250	12500	20	30

Table 2. MIC and MBC (ppm) of antimicrobials against *Salmonella* Enteritidis H4267 at pH 4-7.

pH	Sorbic acid		Benzoic acid		Sodium benzoate		Propionic acid		Sodium propionate		Parabens		Lauric arginate	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4.0	40	100	40	100	40	200	80	400	100	200	100	400	5	30
5.0	200	400	200	800	300	400	200	1560	625	20000	800	1560	45	50
6.0	400	1500	3125	6250	1500	4500	6000	16000	5000	>20000	1560	3120	25	35
7.0	1500	4000	12500	25000	6000	7000	50000	>50000	20000	>20000	1560	3120	20	25

Table 3. Fractional inhibitory concentration index (FICI) of lauric arginate in combination with other antimicrobials at pH 5.0-7.0.

Bacteria	pH	With parabens		With sorbic acid		With benzoate	
		FICI	Mode*	FICI	Mode*	FICI	Mode*
L. monocytogenes Scott A	5.0	0.75	PS	1.25	I	1.25	I
	6.0	0.5	S	1.25	I	1	A
	7.0	0.5	S	1	A	1.25	I
Salmonella Enteritidis H4267	5.0	1	A	1	A	1	A
	6.0	0.5	S	0.75	PS	0.75	PS
	7.0	0.75	PS	1.25	I	1	A

*PS: partially synergistic (FICI = 0.5-0.75); S: synergistic (FICI <0.5); A: additive (FICI = 0.75-1); I: indifferent (FICI = 1-4).

Table 4. Approximate analysis of commercial coatings.

Coating code	pH	Moisture (%)	Total solids (%)	Ash (%)	Fat (%)
EXC 7037	8.7	74.22± 0.71	26.27± 0.01	2.97±.0.23	68.97
EXC 6057	10.46	74.63± 1.15	25.37± 1.15	3.10±0.37	63.97
EXC 6095	9.2	70.96± 2.67	29.03± 2.67	3.55±0.50	65.88
EXC 7036	10.39	75.85± 0.10	24.14± 0.10	3.32±0.89	23.05

Table 5. Appearance of the commercial coatings after adjusting to pH 6.0 and dissolving with 1% antimicrobial.

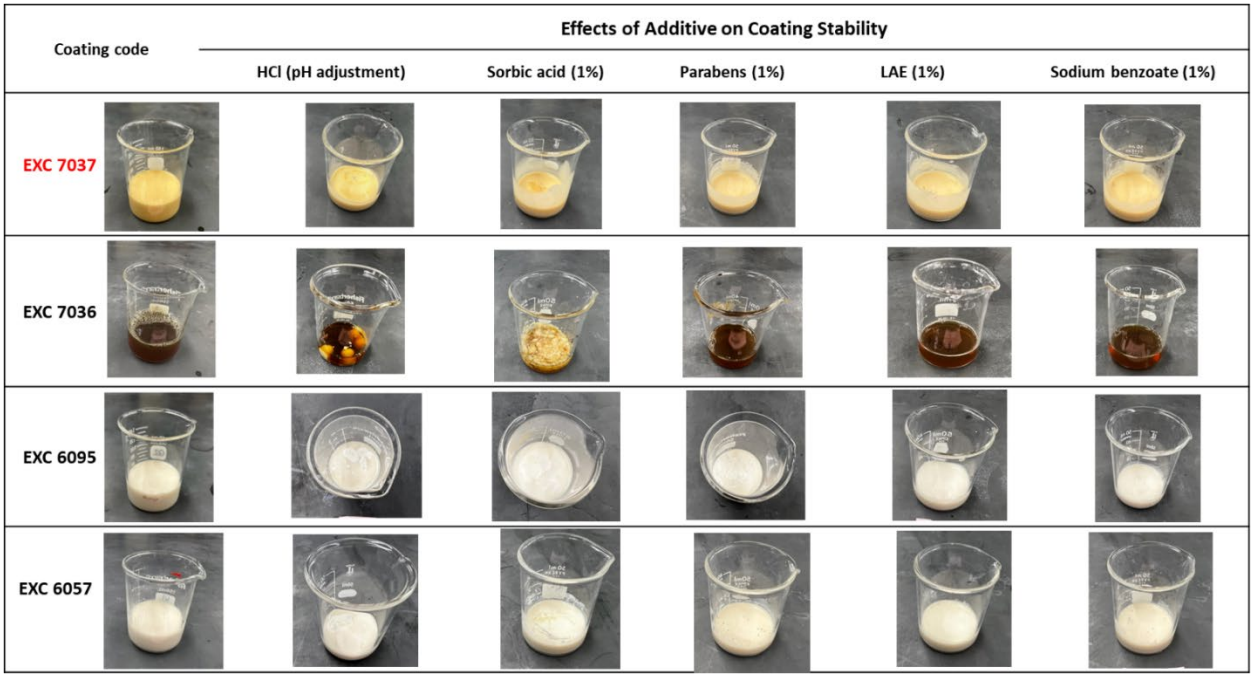


Table 6. Log reduction of *Salmonella* Enteritidis H4267 and *L. monocytogenes* Scott A in the EXC 7037 coating supplemented with lauric arginate (LAE) combined with different antimicrobials or methyl- and propyl (2:1, w:w) parabens at different concentrations, with and without adjusting pH to 6.0, after incubation at room temperature (~21°C) for 90 min.

Coating pH	Antimicrobials (ppm)	Reduction (log CFU/mL)	
		Salmonella Enteritidis	*L. monocytogenes*
6.0	LAE (200) + Sorbic acid (5000)	3.42±0.07	0.70±0.04
	LAE (200) + Parabens (5000)	0.02±0.005	0.14±0.03
	LAE (200) + Benzoate (5000)	0.18±0.01	1.04±0.02
	LAE (200) + Parabens (7000)	0.60±0.18	0.13±0.02
	LAE (200) + Benzoate (7000)	0.24±0.07	1.11±0.01
8.7 (unadjusted)	LAE (1000) + Sorbic acid (10,000)	0.24±0.05	0.09±0.04
	LAE (2,000) + Sorbic acid (20,000)	0.69±0.24	0.28±0.02
	Parabens (5000)	0.23±0.14	1.27±0.08
	Parabens (7500)	0.70±0.3	1.41±0.03
	Parabens (10,000)	5.45±0.12	2.95±0.07
	Parabens (15,000)	7.05±0.33	3.03±0.04
	Parabens (20,000)	8.29±0.03	8.18±0.11

Table 7. The population of native fungi and reduction of *Salmonella* or *L. monocytogenes* cocktail inoculated on peaches with and without spraying with the EXC 7037 coating adjusted to pH 6.0 and supplemented with no, low level (0.05% lauric arginate and 0.5% sorbic acid), or high level (0.1% lauric arginate and 1.0 % sorbic acid) antimicrobials after 5-day storage at 21°C.*

Coating conditions	Reduction (log CFU/fruit)		Native fungi (log CFU/fruit)
	Salmonella	*L. monocytogenes*	
Coating with high antimicrobial level	1.26^{b}±0.66	0.49^{b}±0.34	4.06^{b}±0.60
Coating with low antimicrobial level	1.21^{b}±0.31	0.35^{b}±0.24	4.4^{b}±0.60
Coating with no antimicrobials	0.97^{b}±0.16	0.28^{b}±0.23	5.3^{a}±0.50
Uncoated	2.04^{a}±0.45	1.43^{a}±0.30	4.07^{b}±0.36

*Mean values (n = 12) with different superscript letters in the same column differ significantly ($p < 0.05$).

Table 8. The population of native fungi and reduction of *Salmonella* or *L. monocytogenes* cocktail inoculated on peaches with and without spraying with the EXC 7037 coating adjusted to pH 6.0 and supplemented with no, low level (0.05% lauric arginate and 0.5% sorbic acid), or high level (0.1% lauric arginate and 1.0 % sorbic acid) antimicrobials after 20-day storage at 0°C.*

Coating conditions	Reduction (log CFU/fruit)		Native fungi (log CFU/fruit)
	Salmonella	*L. monocytogenes*	
Coating with high antimicrobial level	2.26^{a}±0.39	2.71^{b}±0.49	3.93^{b}±0.40
Coating with low antimicrobial level	1.47^{b}±0.37	2.35^{b}±0.37	4.14^{b}±0.52
Coating with no antimicrobials	0.62^{c}±0.33	2.49^{b}±0.34	4.74^{a}±0.40
Uncoated	2.07^{a}±0.30	4.24^{a}±0.56	3.76^{b}±0.40

*Mean values (n = 12) with different superscript letters in the same column differ significantly ($p < 0.05$).

Table 9. Total soluble solids (TSS), titratable acidity (TA, based on %malic acid), pH, and weight loss of peaches with and without spraying with the EXC 7037 coating adjusted to pH 6.0 and supplemented with no, low level (0.05% lauric arginate and 0.5% sorbic acid), or high level (0.1% lauric arginate and 1.0 % sorbic acid) antimicrobials during 5-day storage at 21°C.*

Coating conditions	Storage time (days)	TSS (°Brix)	TA (%)	pH	Weight loss (%)
Coating with high antimicrobial level	0	11.5^{c}±0.54	7.0^{a}±1.45	3.8^{b}±0.1	-
	3	11.8^{c}±0.38	6.1^{a}±0.98	4.4^{a}±0.11	9.53^{e}±1.07
	5	14.1^{b}±1.31	6.1^{a}±1.86	4.2^{a}±0.28	15.71^{c}±.55
Coating with low antimicrobial level	0	11.5^{c}±0.54	7.0^{a}±1.45	3.8^{b}±0.10	-
	3	$12.4^{b,c}$±0.98	5.8^{a}±1.24	4.2^{a}±0.10	$11.57^{d,e}$±2.02
	5	$12.7^{b,c}$±0.58	8.2^{a}±1.55	$4.0^{a,b}$±0.10	$18.91^{b,c}$±3.22
Coating with no antimicrobials	0	11.5^{c}±0.54	7.0^{a}±1.45	3.8^{b}±0.10	-
	3	$12.7^{b,c}$±0.97	7.0^{a}±1.25	4.2^{a}±0.18	9.54^{e}±0.91
	5	$13.2^{b,c}$ ±0.60	7.2^{a}±1.24	$4.1^{a,b}$±0.07	$15.27^{c,d}$±0.58
Uncoated	0	11.5^{c}±0.54	7.0^{a}±1.45	3.8^{b}±0.10	-
	3	14.2^{b} ±1.05	6.5^{a}±1.5	4.2^{a}±0.14	19.74^{b}±2.36
	5	17.3^{a}±1.76	8.3^{a}±2.01	$4.1^{a,b}$±0.13	31.09^{a}±2.97

*Mean values (n = 12) with different superscript letters in the same column differ significantly ($p < 0.05$).

Table 10. Total soluble solids (TSS), titratable acidity (TA, based on %malic acid), pH, and weight loss of peaches with and without spraying with the EXC 7037 coating adjusted to pH 6.0 and supplemented with no, low level (0.05% lauric arginate and 0.5% sorbic acid), or high level (0.1% lauric arginate and 1.0 % sorbic acid) antimicrobials during 20-day storage at 0°C.*

Coating conditions	Storage time (days)	TSS (°Brix)	TA (%)	pH	Weight loss (%)
Coating with high antimicrobial level	5	$11.7^{b,c}$±1.50	8.1^{a}±1.86	3.7^{f}±0.12	2.38^{h}±0.40
	10	$12.1^{b,c}$±0.96	$7.1^{a,b}$±1.24	$3.8^{d,e,f}$±0.14	4.14^{h}±0.62
	15	$12.6^{b,c}$±1.06	$5.9^{a,b,c}$±1.36	$4.4^{a,b}$±0.16	$8.39^{e,f}$±1.06
	20	$13.5^{b,c}$±0.96	$5.25^{b,c}$±1.43	$3.7^{e,f}$±0.27	11.58^{c}±1.17
Coating with low antimicrobial level	5	$12.0^{b,c}$±0.17	$7.4^{a,b}$±1.15	$3.8^{d,e,f}$±0.07	2.70^{h}±0.42
	10	$12.8^{b,c}$±1.14	$6.9^{a,b,c}$±1.31	$3.9^{c,d,e,f}$±0.07	$4.60^{g,h}$±0.77
	15	$11.8^{b,c}$±1.09	$5.4^{a,b,c}$±0.78	4.3^{a}±0.14	$8.88^{d,e}$±149
	20	$12.7^{b,c}$±1.14	$5.92^{a,b,c}$±1.15	$3.9^{d,e,f}$±0.17	$12.08^{c,d}$±2.07
Coating with no antimicrobials	5	11.6^{c}±0.85	$7.8^{a,b}$±1.09	$3.9^{c,d,e,f}$±0.08	2.31^{h}±0.11
	10	11.5^{c}±0.41	$7.8^{a,b}$±1.62	$3.9^{c,d,e,f}$±0.06	4.14^{h}±0.27
	15	$13.2^{b,c}$±1.13	4.2^{c}±0.69	4.4^{a}±0.15	$8.57^{e,f}$±0.54
	20	$12.2^{b,c}$±0.41	$5.14^{b,c}$±0.54	$4.0^{b,c,d}$±0.07	$12.21^{c,d}$±0.74
Uncoated	5	$12.06^{b,c}$±1.17	8.2^{a}±0.81	$3.8^{d,e,f}$±0.06	$8.03^{f,g}$±1.11
	10	$13.4^{b,c}$±1.39	$6.9^{a,b,c}$±1.62	$4.0^{b,c,d,e}$±0.16	14.78^{c}±1.83
	15	$14.1^{a,b}$±1.66	$7.8^{a,b}$±2.49	$4.1^{a,b,c}$±0.15	30.24^{b}±3.75
	20	16.2^{a}±1.39	$5.47^{a,b,c}$±0.89	$4.2^{a,b,c}$±0.10	40.69^{a}±4.11

*Mean values (n = 12) with different superscript letters in the same column differ significantly ($p < 0.05$).

Table 11. Color parameters of peaches with and without spraying with the EXC 7037 coating adjusted to pH 6.0 and supplemented with no, low level (0.05% lauric arginate and 0.5% sorbic acid), or high level (0.1% lauric arginate and 1.0 % sorbic acid) antimicrobials during 5-day storage at 21°C.[#]

Storage time (days)	Coating conditions	*L**	*a**	*b**
0	Coating with high antimicrobial level	62.9[a]±7.7	25.1[a]±7.5	47.5[a]±8.3
	Coating with low antimicrobial level	63.8[a]±9.1	22.6[a]±9.9	45.9[a]±8.3
	Coating with no antimicrobials	60.5[a]±9.2	19.9[a]±8.0	45.7[a]±8.1
	Uncoated	61.5[a]±8.1	20.6[a]±7.8	42.7[a]±9.1
3	Coating with high antimicrobial level	59.9[a]±13.7	23.8[a]±4.4	46.4[a]±15.5
	Coating with low antimicrobial level	63.4[a]±12.1	23.0[a]±9.8	48.0[a]±12.0
	Coating with no antimicrobials	58.2[a]±11.2	26.9[a]±8.7	44.4[a]±13.1
	Uncoated	64.1[a]±9.2	25.3[a]±5.7	47.1[a]±10.7
5	Coating with high antimicrobial level	58.4[a]±9.5	27.7[a]±6.8	45.8[a]±11.0
	Coating with low antimicrobial level	61.4[a]±8.5	23.3[a]±6.4	50.2[a]±9.1
	Coating with no antimicrobials	61.7[a]±7.4	25.7[a]±6.3	48.4[a]±10.4
	Uncoated	61.6[a]±6.4	26.3[a]±4.8	43.6[a]±8.6

[#]Mean values (n = 12) with different superscript letters in the same column differ significantly ($p < 0.05$).

Table 12. Color parameters of peaches with and without spraying with the EXC 7037 coating adjusted to pH 6.0 and supplemented with no, low level (0.05% lauric arginate and 0.5% sorbic acid), or high level (0.1% lauric arginate and 1.0 % sorbic acid) antimicrobials during 20-day storage at 0°C. [#]

Storage time (days)	Coating conditions	*L**	*a**	*b**
5	Coating with high antimicrobial level	59.6[a]±12.0	26.3[a]±9.5	44.6[a]±10.7
	Coating with low antimicrobial level	63.4[a]±12.1	23.0[a]±9.8	48.0[a]±12.0
	Coating with no antimicrobials	64.4[a]±9.50	22.0[a]±7.8	50.0[a]±9.0
	Uncoated	60.6[a]±12.5	26.1[a]±8.3	42.8[a]±14.4
10	Coating with high antimicrobial level	61.6[a]±13.1	24.4[a]±7.2	45.8[a]±13.3
	Coating with low antimicrobial level	62.7[a]±12.3	25.3[a]±7.1	46.9[a]±10.4
	Coating with no antimicrobials	64.0[a]±13.0	23.7[a]±7.9	47.6[a]±13.0
	Uncoated	65.5[a]±11.0	23.8[a]±7.9	44.9[a]±11.6
15	Coating with high antimicrobial level	60.1[a]±11.8	25.3[a]±8.6	42.9[a]±11.9
	Coating with low antimicrobial level	60.4[a]±13.0	25.6[a]±9.2	44.5[a]±12.1
	Coating with no antimicrobials	64.5[a]±11.4	23.9[a]±10.6	49.3[a]±11.1
	Uncoated	64.8[a]±10.4	23.1[a]±6.6	45.7[a]±10.9
20	Coating with high antimicrobial level	61.3[a]±13.4	23.4[a]±9.4	46.0[a]±12.3
	Coating with low antimicrobial level	56.3[a]±13.8	26.9[a]±7.9	41.3[a]±13.7
	Coating with no antimicrobials	58.0[a]±15.1	24.6[a]±9.5	42.5[a]±15.6
	Uncoated	64.4[a]±11.1	21.5[a]±7.0	43.7[a]±12.2

[#]Mean values (n = 12) with different superscript letters in the same column differ significantly ($p < 0.05$).

Table 13. The population of native fungi and reduction of *Salmonella* or *L. monocytogenes* cocktail inoculated on peaches with and without spraying with the EXC 7037 coating supplemented with parabens after 20-day storage at 0°C.*

Coating conditions	Reduction (log CFU/fruit)		Native fungi (log CFU/fruit)
	Salmonella	*L. monocytogenes*	
Parabens, 2% w/v	3.05^{a}±0.32	1.95^{b}±0.19	4.33^{a}±0.17
Parabens, 1% (w/v)	1.74^{b}±0.22	1.92^{b}±0.17	4.54^{a}±0.48
No antimicrobials	1.24^{b}±0.27	1.41^{c}±0.28	4.57^{a}±0.39
Uncoated	1.06^{c}±0.22	2.92^{a}±0.10	4.13^{a}±0.19

*Mean values (n = 12) with different superscript letters in the same column differ significantly ($p < 0.05$).

Table 14. Color parameters and weight loss of peaches with and without spraying with the EXC 7037 coating supplemented with parabens during 20-day storage at 0°C.#

Coating conditions	Storage time (days)	L*	a*	b*	Weight loss (%)
Parabens, 2% w/v	0	40.1^{a}±8.8	29.6^{a}±5.2	23.9^{a}±10.0	*
Parabens, 1% w/v		38.2^{a}±6.8	32.4^{a}±5.5	22.8^{a}±9.8	*
No antimicrobials		37.1^{a}±6.3	32.1^{a}±3.6	20.1^{a}±7.8	*
Uncoated		40.4^{a}±6.2	31.4^{a}±3.3	21.4^{a}±8.2	*
Parabens, 2% w/v	5	36.8^{a}±5.0	28.4^{a}±9.2	17.5^{a}±6.9	1.19^{f}±0.28
Parabens, 1% w/v		37.4^{a}±6.7	28.5^{a}±6.7	17.6^{a}±8.8	1.68^{f}±0.29
No antimicrobials		36.1^{a}±6.5	29.6^{a}±6.9	18.4^{a}±8.6	1.38^{f}±0.33
Uncoated		42.3^{a}±9.3	28.5^{a}±5.4	24.9^{a}±11.2	1.62^{f}±0.25
Parabens, 2% w/v	10	36.0^{a}±6.2	30.4^{a}±6.3	20.3^{a}±8.8	3.94^{e}±0.53
Parabens, 1% w/v		35.4^{a}±7.1	33.1^{a}±5.0	22.7^{a}±10.2	4.02^{e}±0.38
No antimicrobials		33.3^{a}±9.8	29.0^{a}±6.2	22.8^{a}±11.4	3.76^{e}±0.59
Uncoated		36.4^{a}±7.9	32.6^{a}±5.8	21.5^{a}±9.6	4.03^{e}±0.53
Parabens, 2% w/v	15	31.7^{a}±4.2	27.9^{a}±6.2	14.7^{a}±6.1	$4.51^{c,d,e}$±0.60
Parabens, 1% w/v		36.0^{a}±9.7	31.0^{a}±6.2	25.3^{a}±11.5	$4.53^{c,d,e}$±0.46
No antimicrobials		35.9^{a}±9.6	30.8^{a}±8.3	25.7^{a}±12.4	$4.17^{d,e}$±0.62
Uncoated		36.8^{a}±4.3	35.2^{a}±3.8	22.3^{a}±5.5	$4.62^{b,c,d,e}$±0.50
Parabens, 2% w/v	20	40.4^{a}±9.6	32.6^{a}±5.2	30.2^{a}±10.7	$5.78^{a,b}$±0.75
Parabens, 1% w/v		34.6^{a}±6.4	31.8^{a}±6.3	19.3^{a}±9.3	$5.54^{a,b,c}$±0.63
No antimicrobials		34.8^{a}±9.2	29.8^{a}±8.3	21.7^{a}±11.0	$5.24^{a,b,c,d}$±0.84
Uncoated		36.0^{a}±6.9	31.7^{a}±6.3	21.3^{a}±9.1	5.87^{a}±0.79

#Mean values (n = 12) with different superscript letters in the same column differ significantly ($p < 0.05$).

Table 15. The population of native fungi and reduction of *Salmonella* or *L. monocytogenes* cocktail inoculated on peaches with and without spraying first with parabens and then the EXC 7037 coating after 20-day storage at 0°C.*

Coating conditions	Reduction (log CFU/fruit)		Native fungi (log CFU/fruit)
	Salmonella	*L. monocytogenes*	
Parabens, 2% w/v	3.84^{a}±0.70	2.19^{b}±0.23	2.86^{b}±0.25
Parabens, 1% w/v	3.48^{a}±0.27	2.04^{b}±0.20	3.02^{b}±0.17
No antimicrobials	1.29^{b}±0.27	1.32^{b}±0.18	3.94^{a}±0.12
Uncoated	1.02^{b}±0.28	2.46^{a}±0.25	3.63^{a}±0.27

*Mean values (n = 12) with different superscript letters in the same column differ significantly ($p < 0.05$).

Table 16. Color parameters of peaches with and without spraying first with parabens and then the EXC 7037 coating during 20-day storage at 0°C.#

Coating conditions	Storage time (days)	L*	a*	b*	Weight loss (%)
Parabens, 2% w/v	0	57.1^{a}±7.8	$25.52^{a,b}$±7.5	43.7^{a}±7.3	*
Parabens, 1% w/v		52.7^{a}±11.5	$29.4^{a,b}$±6.3	41.0^{a}±13.7	*
No antimicrobials		42.7^{a}±9.8	$30.2^{a,b}$±2.3	30.1^{a}±11.3	*
Uncoated		51.3^{a}±10	$26.4^{a,b}$±3.2	34.5^{a}±11.0	*
Parabens, 2% w/v	5	45.2^{a}±12.2	$31.1^{a,b}$±5.6	38.9^{a}±13.9	$1.51^{g,h,i}$±0.36
Parabens, 1% w/v		42.0^{a}±10.6	33.3^{a}±4.8	35.2^{a}±10.8	$1.36^{h,i}$±0.21
No antimicrobials		46.1^{a}±9.9	$29.8^{a,b}$±2.9	31.5^{a}±11.7	1.26^{i}±0.17
Uncoated		45.0^{a}±8.2	$28.2^{a,b}$±4.0	26.7^{a}±8.3	1.23^{i}±0.21
Parabens, 2% w/v	10	49.3^{a}±9.9	$28.5^{a,b}$±5.2	33.9^{a}±9.7	$2.09^{f,g}$±0.38
Parabens, 1% w/v		43.4^{a}±10.8	$25.9^{a,b}$±5.2	23.5^{a}±12.7	$1.78^{g,h,i}$±0.12
No antimicrobials		45.1^{a}±10.1	$33.0^{a,b}$±9.7	30.6^{a}±11.9	$1.79^{g,h,i}$±0.12
Uncoated		51.6^{a}±10.1	23.1^{b}±8.5	33.9^{a}±13.9	$1.98^{f,g,h}$±0.26
Parabens, 2% w/v	15	46.7^{a}±2.8	$31.1^{a,b}$±1.0	32.0^{a}±4.7	$2.89^{c,d,e}$±0.43
Parabens, 1% w/v		38.6^{a}±9.2	$27.9^{a,b}$±2.3	21.1^{a}±10.2	$2.53^{e,f}$±0.17
No antimicrobials		45.6^{a}±8.2	$31.1^{a,b}$±2.4	30.4^{a}±8.7	$2.61^{d,e,f}$±0.15
Uncoated		48.0^{a}±9.9	$26.5^{a,b}$±2.6	30.3^{a}±12.6	$3.20^{c,d}$±0.49
Parabens, 2% w/v	20	40.9^{a}±8.8	$27.9^{a,b}$±6.9	23.7^{a}±10.6	$3.86^{a,b}$±0.53
Parabens, 1% w/v		44.9^{a}±9.0	$29.3^{a,b}$±4.8	25.2^{a}±10.3	$3.47^{b,c}$±0.24
No antimicrobials		43.9^{a}±10.7	$26.4^{a,b}$±6.7	23.3^{a}±12.0	$3.45^{b,c}$±0.13
Uncoated		47.2^{a}±8.4	$25.2^{a,b}$±4.7	23.3^{a}±11.4	4.12^{a}±0.53

#Mean values (n = 12) with different superscript letters in the same column differ significantly ($p < 0.05$).

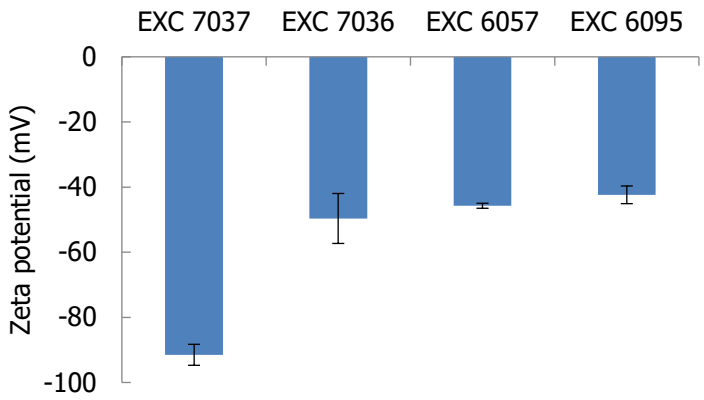


Fig. 1. Zeta potential of the commercial coatings as received.

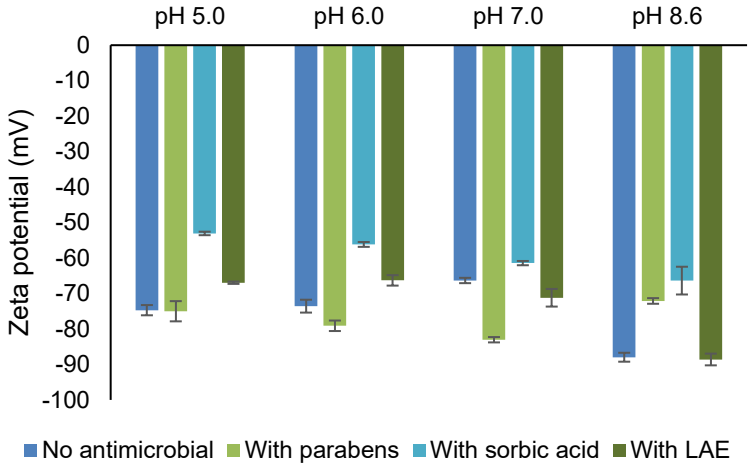


Fig. 2. Zeta potential of the EXC 7037 coating with antimicrobials (1%) at different pH.

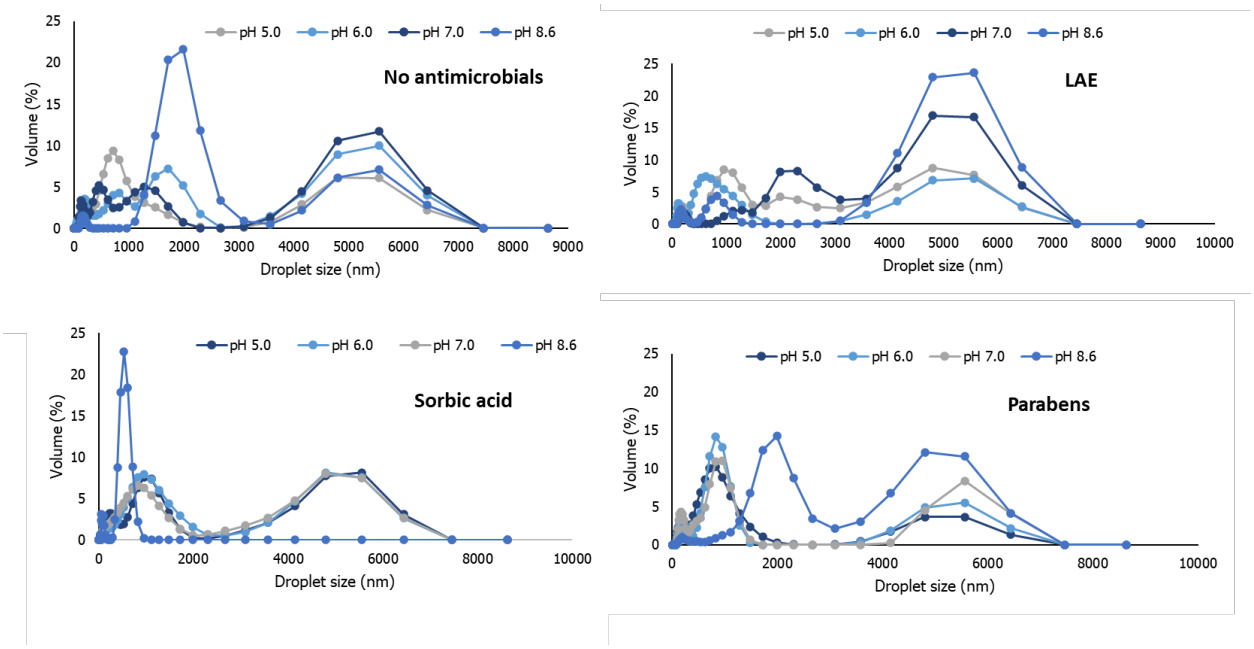


Fig. 3. Droplet size of the EXC 7037 coating with antimicrobials (1%) at different pH.

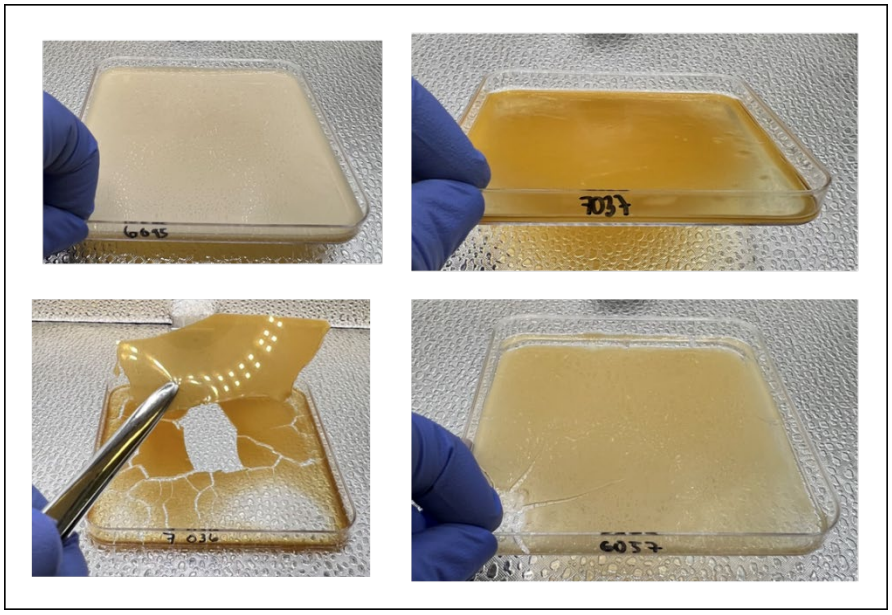
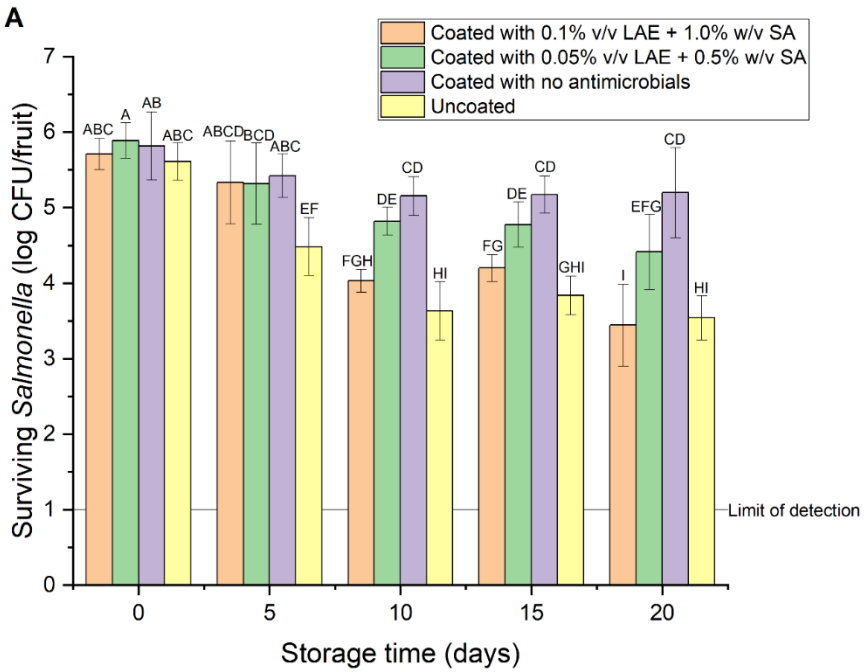


Fig. 4. Films cast from commercial coatings after drying for 24 h.



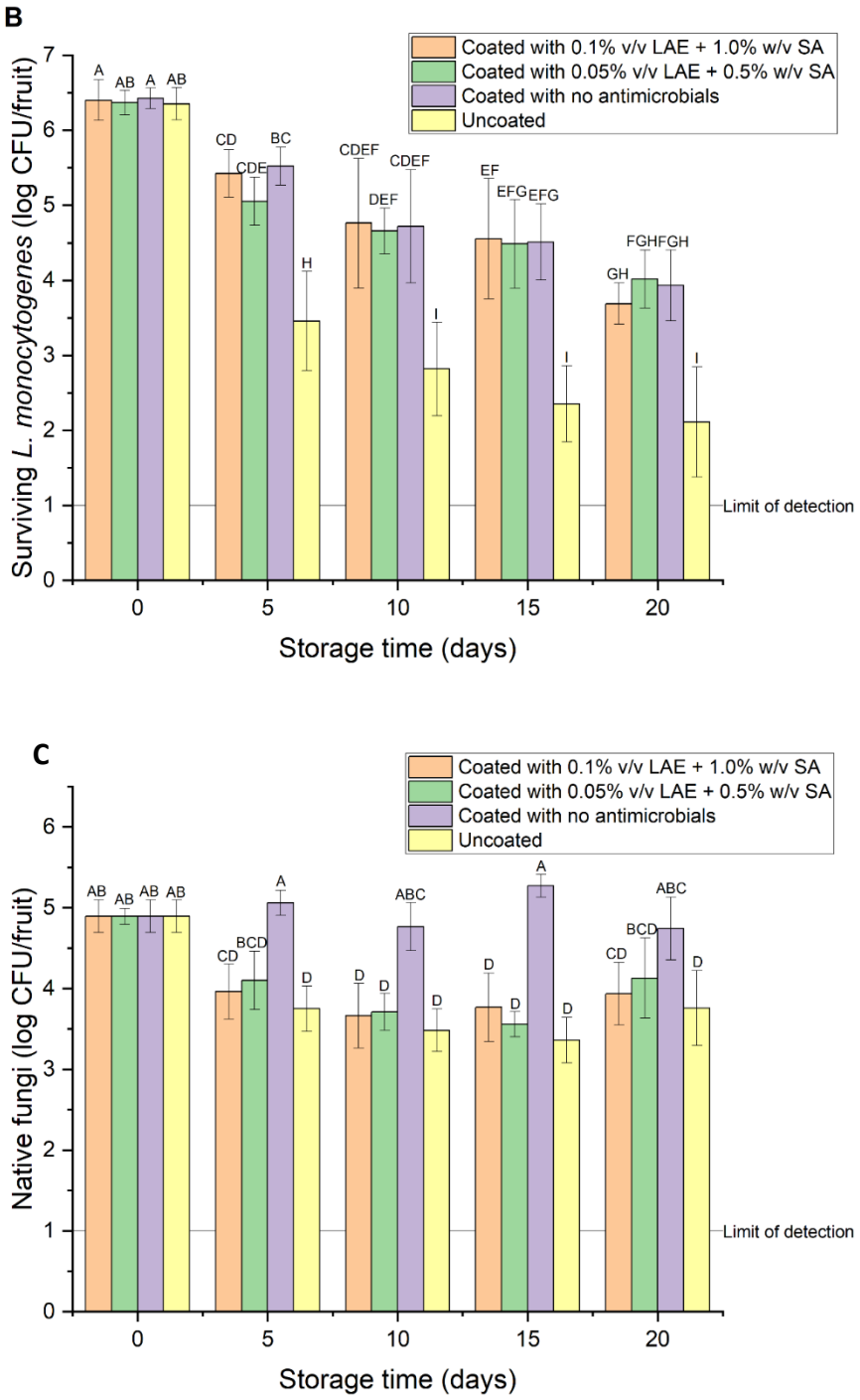


Fig. 5. Survival of *Salmonella* (A) or *L. monocytogenes* (B) cocktail inoculated on peaches and native fungi (C) without and with spraying with the EXC 7037 coating adjusted to pH 6.0 and supplemented with and without lauric arginate (LAE) and sorbic acid (SA) during 20-day storage at 0°C. Error bars represent SD (n = 12). Mean values with different uppercase letters above columns denote significant differences ($p < 0.05$) among all data points.

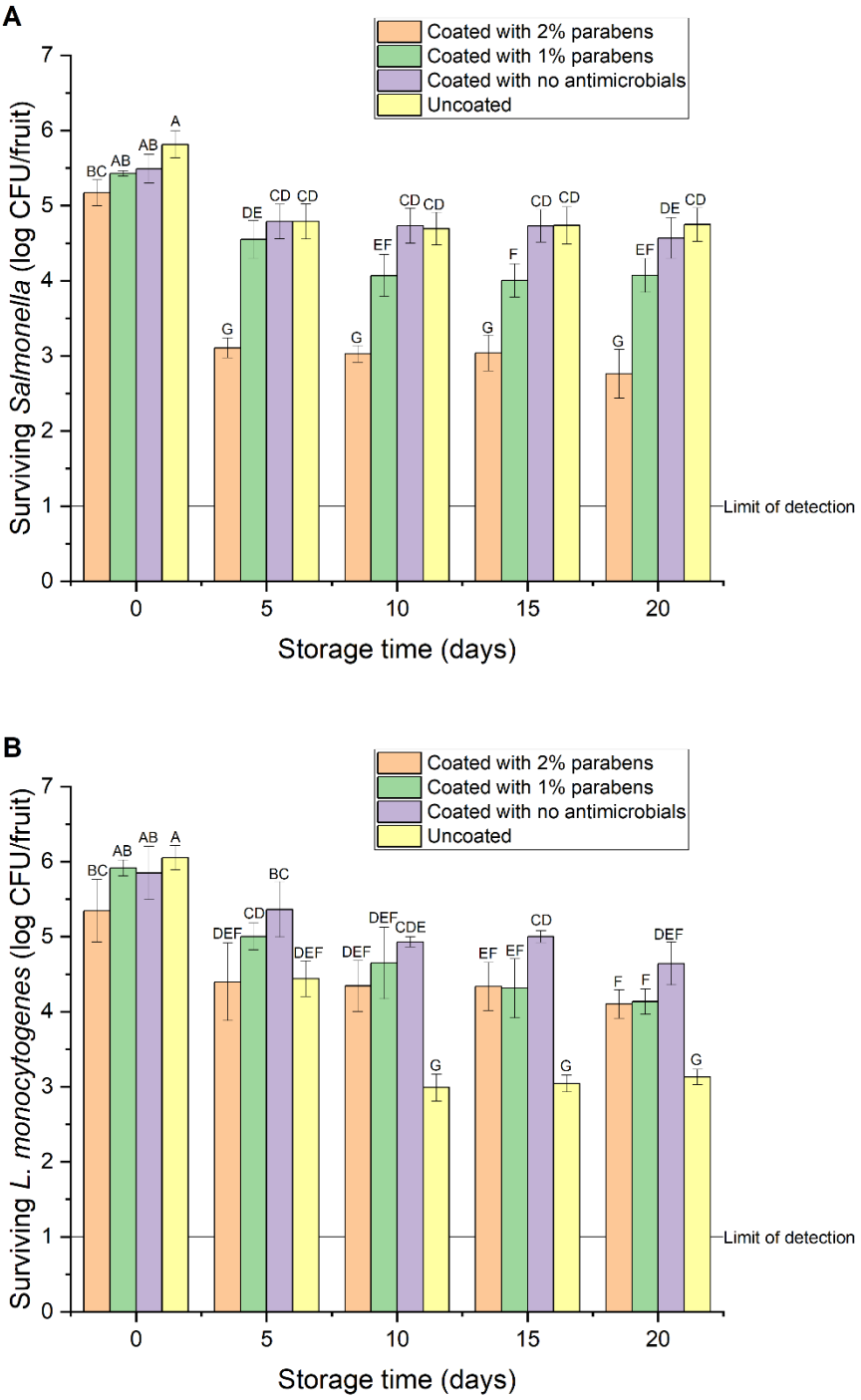


Fig. 6. Survival of *Salmonella* (A) or *L. monocytogenes* (B) cocktail inoculated on peaches without and with spraying with the EXC 7037 coating supplemented with and without 1.0% or 2.0% w/v parabens during 20-day storage at 0°C. Error bars represent SD (n = 12). Mean values with different uppercase letters above columns denote significant differences ($p < 0.05$) among all data points.

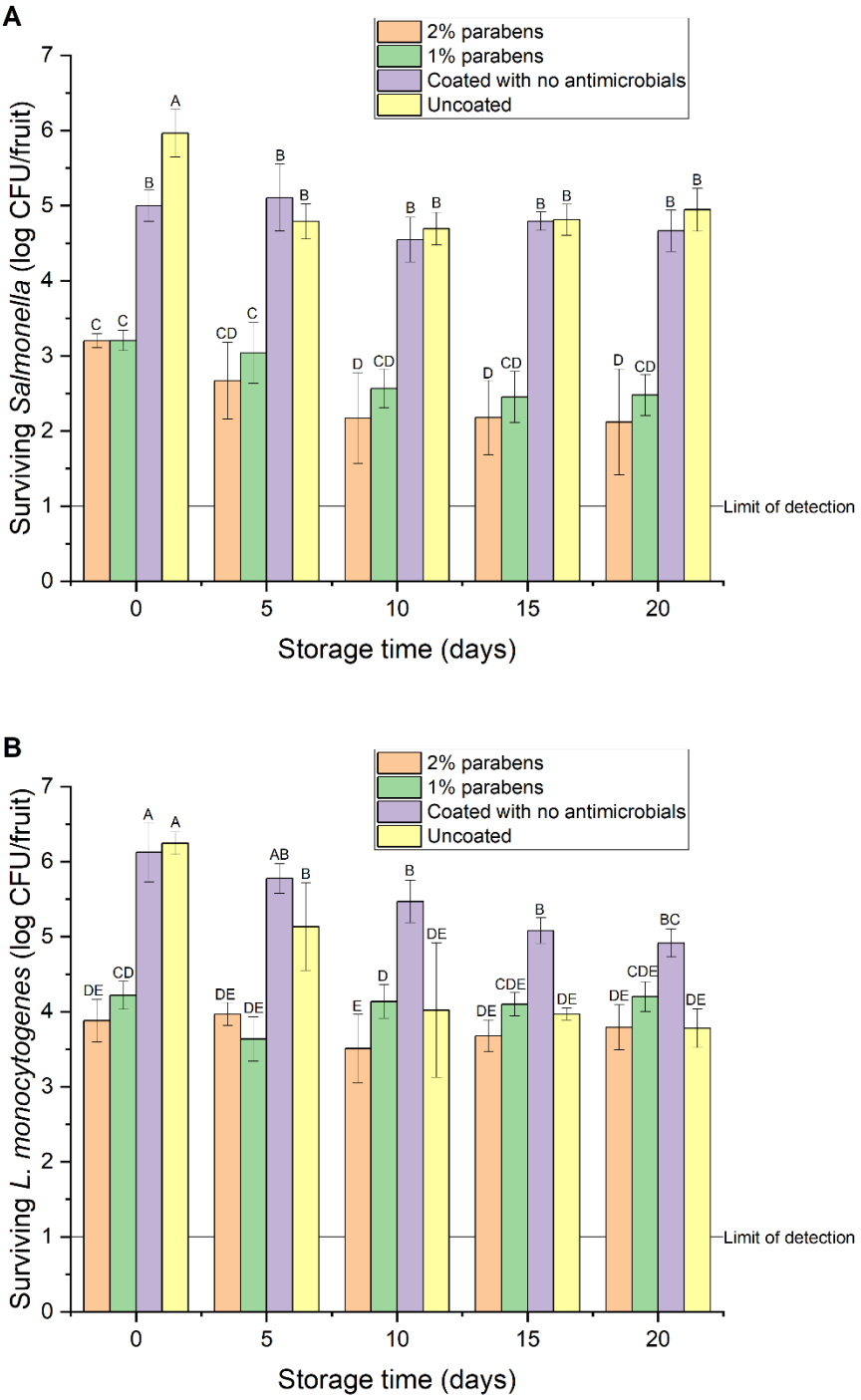


Fig. 7. Survival of *Salmonella* (A) or *L. monocytogenes* (B) cocktail inoculated on peaches without and with first spraying with 1.0% or 2.0% w/v parabens followed by applying the EXC 7037 coating during 20-day storage at 0°C. Error bars represent SD (n = 12). Mean values with different uppercase letters above columns denote significant differences ($p < 0.05$) among all data points.