# Sustainable Food Technology



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# Effects of supplementing lauric arginate and sorbic acid in a commercial coating on pathogen survival and quality of fresh peaches

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Commercial coatings for stone fruits improve the quality but have limitations in controlling incident foodborne pathogens during storage. The aim of this study was to evaluate the pathogen survival and quality of fresh peaches sprayed with a commercial stone fruit coating, supplemented with low level (0.05% v/v lauric arginate - LAE and 0.5% w per v sorbic acid) and high level (0.1% v/v LAE and 1.0% w per v sorbic acid) antimicrobials. The alkaline coating was adjusted to pH 6.0 based on an observed partial synergistic effect between LAE and sorbic acid. Fresh peaches were inoculated with Salmonella or Listeria monocytogenes cocktails and then sprayed with the coating. The fruits in open trays were stored in a walk-in refrigerator set at 0 °C and 85% relative humidity for 20 days or at 21 °C for 5 days. Quality evaluation followed the same protocol using uninoculated peaches. Gradual reductions in viable bacteria were observed for both pathogens. After storage at 0 °C, the control, low-level, and high-level antimicrobial treatments achieved a respective reduction of 0.41  $\pm$  0.54, 1.20  $\pm$  0.71, and 2.17  $\pm$  0.53 log CFU per fruit for Salmonella, while the log reductions (2.34-2.66 log CFU per fruit) were similar for L. monocytogenes. Pathogen counts were similar in the coated peaches throughout storage at 21 °C. The antimicrobials in the coating reduced native fungi in peaches by 0.5-1.0 log CFU per fruit after storage at either temperature and did not significantly impact the total soluble solids, titratable acidity, pH, and weight loss of the peaches during storage. The results demonstrate that LAE and sorbic acid are options to control Salmonella in fresh peaches, but additional strategies are needed to inhibit L. monocytogenes.

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#### Sustainability spotlight

The sustainable production of fresh produce requires technologies that reduce waste and loss due to microbial spoilage and quality deterioration and prevent recalls due to contamination of foodborne pathogens causing illnesses. For stone fruits, coatings are applied to improve quality, but fungicides used in commercial coatings are ineffective against foodborne bacterial pathogens and are not label friendly. By investigating the inhibition of *Salmonella*, *Listeria monocytogenes*, and native fungi on fresh peaches using generally recognized as safe food preservatives incorporated in a commercial stone fruit coating, the present study contributes to the sustainable production of fresh produce.

# 1. Introduction

Peach (*Prunus persica* L.) is a climacteric stone fruit and the second most important temperate fruit crop worldwide in terms of production, after apple. Peaches have a limited shelf life due to postharvest weight loss, mechanical damage, and potential presence of pathogenic bacteria and fungi. Fruit spoilage is often associated with the skin, potentially leading to browning,

off-flavors, texture breakdown, and pathogen contamination.<sup>3</sup> The stone fruit industry uses two main strategies to maintain the quality of the peaches, cold condition storage (0–5 °C and 80–95% relative humidity – RH)<sup>4</sup> and coating the fruit with food grade materials by dipping, spraying, brushing, or dripping.<sup>2</sup> Edible coatings form a semi-permeable barrier that reduces respiration and water loss while helping retain firmness and volatile compounds.<sup>5</sup> However, most coatings do not inherently prevent the growth of incident pathogens, but stone fruits have been linked to several multistate outbreaks. *Salmonella* Enteritidis, for example, contaminated peaches in summer 2020, causing 101 illnesses in 17 states; more recently, in November 2023, a *Listeria* outbreak in multiple states was linked to whole peaches, nectarines, and plums, causing 11 illnesses, including 10 hospitalizations and 1 death.<sup>6,7</sup> Hence, strategies such as

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incorporating antimicrobials are needed to control microbial safety and sustainable production of stone fruits.

Lauric arginate (LAE, ethyl-Nα-lauroyl-L-arginate hydrochloride) is a generally recognized as safe (GRAS) antimicrobial preservative with an acceptable daily intake (ADI) of 0.5 mg kg<sup>-1</sup> body weight as established by the European Food Safety Authority (EFSA).8 LAE has activities against various microorganisms in microbiological growth media and food matrices when used alone or in combination with other antimicrobials.9 On the other hand, sorbic acid is a weak organic acid typically used as a single preservative. 10 Sorbic acid has an ADI of 11 mg kg<sup>-1</sup> body weight per day as established by the EFSA.<sup>11</sup> The potential synergy of sorbic acid with other antimicrobial agents such as LAE and their addition to commercial coatings may offer new preservation methods for stone fruits. Therefore, the objective of this study was to evaluate the survival of pathogenic bacteria and native fungi on fresh peaches, as well as the impact on the quality, after spraying with a commercial stone fruit coating supplemented with LAE and sorbic acid followed by storage at 0 or 21 °C.

# 2. Materials and methods

#### 2.1. Produce commodities

Fresh and unwashed peaches (*Prunus persica* L. Batsch) were purchased from Safe Fresh Fruit (Fresno, CA, USA) and stored in a clean and disinfected cold room at 0 °C upon arrival. Peaches with similar size and color, without physiological defects, were chosen for the study. The selected peaches were brushed and washed with deionized water, then dried and stored in aluminum trays.

#### 2.2. Materials

LAE®, containing 15  $\pm$  0.5% w/w LAE, was kindly provided by Vedeqsa Inc (Independence, MO, USA). Sorbic acid (>99% purity) was purchased from Sigma-Aldrich Corp (St. Louis, MO, USA). Broth and agar media were obtained from Thermo Fisher Scientific Inc (Pittsburgh, PA, USA), except for xylose lysine deoxycholate agar (XLD), modified Oxford agar (MOX) and potato dextrose agar (PDA), which were obtained from Becton, Dickinson and Company (Sparks, MD, USA). The commercial stone fruit coating EXC 7037, with a measured pH of 8.7, was provided by Pace International, LLC (Wapato, WA, USA); its composition is proprietary.

### 2.3. Physical properties and fat content of the coating

The droplet size distribution and zeta-potential of the coating, as received (pH 8.7) and after adjustment to pH 6.0 with 2.0 N HCl, were measured with a Zetasizer Nano-ZS instrument (Malvern Instruments Ltd, Worcestershire, UK) following a 10-fold dilution in deionized water. Fat content of the coating was determined using the Soxhlet method following the AOAC official method 960.39.<sup>13</sup>

#### 2.4. Bacterial strains

For *in vitro* assays, *Listeria monocytogenes* Scott A and *Salmonella* Enteritidis ATCC H4267 were tested. For experiments on the peaches, a separate cocktail of *Salmonella* or *L. monocytogenes* was used. A total of five strains were used for the *Salmonella* cocktail, including *Salmonella* Enteritidis (ATCC H4267 and S5-371), *Salmonella* Javiana (S5-406), and *Salmonella* Typhimurium (A4-737, and S5-370). Five strains used for the *L. monocytogenes* cocktail included serotype 1/2A strains 10403S (FSL X1-0001) and Mack (FSL F6-0367), and serotype 4b strains including Scott A, F2365 (FSL R2-0574) and FSL F2-501. All strains were kept at -80 °C in tryptic soy broth (TSB) with 40% v/v of glycerol.

#### 2.5. Culture and inoculum preparation

Bacteria were revived from the glycerol stock by inoculating into TSB for *Salmonella* and brain heart infusion (BHI) broth for *L. monocytogenes*. The strains were incubated for 24 h at 37 °C for *Salmonella* or 32 °C for *L. monocytogenes*. Prior to use, each strain was sub-cultured twice in 10 mL of sterile TSB or BHI broth, with a 24 h interval. To prepare the five-strain *Salmonella* or *L. monocytogenes* cocktail, each strain was combined at an equal volume, centrifuged at 10 000×g for 10 min at 4 °C (Sorvall ST 17R, Thermo Scientific Company, Waltham, MA, USA), washed twice with 0.1% w per v sterile peptone water, and resuspended in 12.5 mL of 0.1% w per v peptone water. The population of the cocktails was about 9 log CFU mL<sup>-1</sup> and was confirmed by spread-plating 0.1 mL on XLD for *Salmonella* or MOX for *Listeria*, followed by incubation for 24 h at 37 °C for *Salmonella* or 32 °C for *L. monocytogenes*. To

#### 2.6. Determination of antimicrobial activity in TSB

The efficacy of the antimicrobials against one individual strain of Salmonella or L. monocytogenes in the growth media (TSB or BHI) adjusted to pH 5.0-7.0 was determined using the microbroth dilution method.17 The stock solutions of LAE (1% v/v) and sorbic acid (10% w v<sup>-1</sup>) were prepared in sterile deionized water, and the pH was adjusted to 5.0, 6.0, and 7.0 with 2.0 N HCl or 0.1 N NaOH. The working antimicrobial solutions were prepared by serially diluting the stock solution in the media pre-adjusted to the same pH. The stock culture was also diluted in the media to approximately 10<sup>6</sup> CFU mL<sup>-1</sup> as the working culture. A 96-microtiter plate was filled with 100 µL of the antimicrobial working solution and 100 μL of the working culture in each well. The 96-microtiter plate was then incubated at 37 °C for Salmonella or 32 °C for L. monocytogenes for 24 h. Bacterial growth was determined by measuring the optical density of each well in a Synergy HT multi-detection microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). 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 ( $\Delta OD_{630nm}$ ). To determine the minimum bactericidal concentration (MBC), 0.1 mL aliquots from each microtiter well showing non- or minimal growth were spread-plated on tryptic soy agar (TSA). After incubation for 48 h at 32 °C or 37 °C,

colonies were counted, and the MBC was defined as the lowest antimicrobial concentration giving ≥3 log decrease in viable cells in comparison to the initial inoculum. Three replicates were performed for each strain and each antimicrobial.

Based on the individual MICs, antimicrobials were also tested when used in combinations using the checkerboard method.18 Briefly, 120 μL of an antimicrobial solution (with up to 45 ppm LAE and up to 200 ppm sorbic acid) and 120 µL of the working culture (ca 10<sup>6</sup> CFU mL<sup>-1</sup>) were added to each well of a 96-well microtiter plate. MICs of antimicrobials in combinations were determined as above, and the fractional inhibitory concentration index (FICI) was calculated as shown in eqn (1). The results were interpreted as synergy (FICI  $\leq$  0.5), partial synergy (0.5 < FICI  $\leq$  0.75), addition (0.75 < FICI < 1.0), indifference (1.0  $\leq$  FICI  $\leq$  4), or antagonism (FICI > 4.0).<sup>19</sup>

$$FICI = \frac{MIC \text{ of } LAE \text{ in combination}}{MIC \text{ of } LAE \text{ alone}} + \frac{MIC \text{ of sorbic acid in combination}}{MIC \text{ of sorbic acid alone}}$$
(1)

#### 2.7. Pathogen survival in the commercial coating

To determine the efficacy of the antimicrobials in the commercial coating dispersion, LAE and sorbic acid were dissolved at various concentrations in the coating, with and without pre-adjusting to pH 6.0 using 2.0 N HCl. Then, 9.0 mL of the coating dispersion with the antimicrobials was inoculated with 1.0 mL of a culture containing  $\sim 9 \log \text{ CFU mL}^{-1}$ , followed by incubation at room temperature (RT, 21 °C) in a shaker at 350 rpm.20 The inoculated coating dispersion was sampled after 0, 10, 30, 60, and 90 min, serially diluted with sterile peptone water (0.1%), and plated in duplicate onto XLD or MOX plates. After incubation for 24 h at 32 °C for Listeria or 37 °C for Salmonella, colonies were counted. The coating with inoculum and no antimicrobials served as positive control, and the coating without inoculum served as a negative control. Two independent biological replicates were used.

#### 2.8. Experimental design for coating peaches

The antimicrobials were dissolved at a low level (0.05% v/v LAE and 0.5% w per v sorbic acid) or high level (0.1% v/v LAE and 1.0% w per v sorbic acid) in the commercial coating dispersion pre-adjusted to pH 6.0 using 2.0 N HCl. The pH 6.0 coating without antimicrobials was evaluated directly as a control, and uncoated peaches were evaluated as another control. After the coating process, all peaches in open trays were stored in a walk-in refrigerator at 0 °C and 85% RH and sampled every 5 days for up to 20 days. Another set of peaches in open trays was stored at 21 °C and 85% RH and sampled on day 0, 3, and 5. The study was repeated two times in different months using two separate batches of peaches, with 6 fruits sampled per day per coating condition (n = 12).

2.8.1. Inoculation of peaches. Peaches were washed, dried, and then spot-inoculated with the bacterial cocktail by placing 200  $\mu L$  of the inoculum, containing  $\sim$ 7 log CFU mL<sup>-1</sup>, at 10 different spots (20 µL each) around the stem.21 The inoculated

peaches were kept at RT in a biosafety hood for 4 h to allow the inoculum to dry. To determine the initial bacterial load, six peaches were analyzed after drying the inoculum.

- 2.8.2. Spray-coating and storage of peaches. The inoculated peaches were each sprayed with the coating using a gravity feed dual-action air-nozzle sprayer equipped with an airbrush compressor (PointZero Model No. Elite-125X, Tamarac, FL, USA) and 0.3 mm nozzle at a pressure of 350 000 Pa. Each peach was sprayed with three pulls (about 1 mL) above the stem at RT at a distance that ensured uniform application on the fruit surface.21 Four hours after drying at RT, 6 peaches were placed on each unsealed sanitized aluminum tray and moved to the refrigerator. Inoculated but uncoated peaches were stored in the same room. Another set of peaches was stored at 21 °C. Peaches were held in unsealed containers to simulate conditions in which fruits are held and transported in large containers.16
- 2.8.3. Microbial analysis of peaches. Each peach was placed in a sterile filter bag (Fisher Scientific), and 50 mL of sterile peptone (0.1% w v<sup>-1</sup>) was added to each bag. Each peach was then hand-massaged for 120 s, and the detached suspension was serially diluted with sterile peptone water (0.1% w v-1) and plated on XLD agar in duplicate to enumerate Salmonella, MOX to enumerate L. monocytogenes22 or PDA to enumerate native yeasts and molds.<sup>23</sup> The plates were incubated for 24 h at 37 °C and 32 ° C for Salmonella and L. monocytogenes, respectively. PDA plates were incubated at RT for 3-5 days. Colonies were counted, and the log CFU per fruit was determined. Log reductions were calculated by referring to the uncoated peaches on day 0.
- 2.8.4. Quality assessment of peaches. Independent peaches without inoculated bacteria were processed and stored as above to evaluate the physicochemical quality attributes. These attributes were tested with only one batch of peaches (n =6) because of the insignificant difference among coating treatments. Peaches were labeled with numbers and weighed at every sampling time. A digital balance was used to assess the percentage of weight loss during storage. Separate peaches were used to determine the pH, titratable acidity (TA), and total soluble solids (TSS) content (oBrix) during storage. Peach puree was prepared by homogenizing freshly sliced flesh at 10 000 rpm for 2 min using a high-speed homogenizer (model IKA T25 digital, IKA-Werke GmbH & Co., Staufen, Germany). The TSS of the puree was assessed with an Atago PR-32 digital Brix refractometer (Atago Co Ltd, Tokyo, Japan). The puree (1 g) after dilution in 100 mL deionized water was used to determine pH and TA. The TA was estimated from titration using 0.1 N NaOH and was reported in malic acid content (%).24

A colorimeter (CR-400, Konica Minolta, Inc., Tokyo, Japan) was used for color measurements of the peach surface. The data were expressed in the CIELAB color space ( $L^*$ ,  $a^*$ , and  $b^*$ ), with the  $L^*$  value ranging from 0 to 100 and  $a^*$  and  $b^*$  values from -128 to 127. Each peach was measured at three different locations.

#### 2.9. Statistical analysis

Bacterial populations were subjected to log<sub>10</sub> transformation before statistical analysis. All results were reported as mean  $\pm$ 

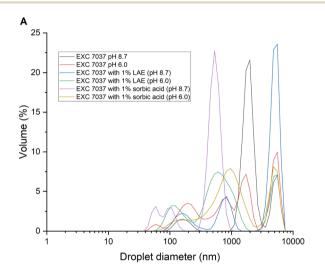
standard deviation (SD). The mean difference was discerned by one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons using Minitab 16 (IBM, Chicago, IL, USA). A p-value of less than 0.05 was considered significant.

# 3. Results and discussion

#### 3.1. Droplet size and zeta-potential of the coating

Fig. 1A shows the droplet size distribution of the coating before (pH 8.7) and after adjusting to pH 6.0. At pH 8.7, the addition of LAE increased the droplet size, while the opposite was the case for sorbic acid. Adjusting the pH to 6.0 increased the droplet size, and the addition of LAE or sorbic acid had a less significant impact on the droplet size than the coating at pH 8.7, showing slight decrease for the portion of droplets with a diameter between 1000 and 3000 nm.

The coating exhibited a highly negative zeta-potential, which became less negative (p < 0.05) when pH was decreased from 8.7 to 6.0 (Fig. 1B). This decrease in the zeta-potential magnitude as



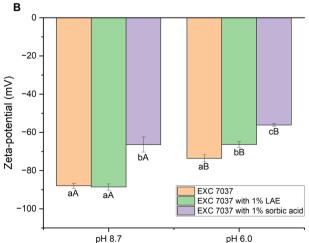


Fig. 1 Droplet size distribution (A) and zeta-potential (B) of the EXC 7037 coating before (pH 8.7) and after adjusting to pH 6.0. Different lowercase letters in (B) show the difference among treatments at the same pH, while different uppercase letters show the difference of the same treatment at different pH (p < 0.05).

pH is reduced is common for compounds containing ionizable groups, such as carboxyl groups, that become less charged after protonation. At pH 8.7, the addition of LAE did not significantly change zeta-potential (p > 0.05). LAE is positively charged and may bridge lipid droplets in the coating, which increases the droplet size (Fig. 1A) but can have a negligible effect on the measured zeta-potential because the bridged droplets with LAE in the center can have the same surface charge density as individual droplets. At pH 6.0, the reduced charge of lipid droplets may reduce the bridging effect of LAE, corresponding to the smaller difference in the droplet size of the coating with and without LAE than at pH 8.7 (Fig. 1A), and the overall zetapotential became less negative (p < 0.05) after addition of LAE (Fig. 1B). On the other hand, sorbic acid lowered (p < 0.05) the zeta-potential of the coating at both pH 8.7 and 6.0 (Fig. 1B), possibly because sorbate increases the ionic strength and the charge screening effect lowers the measured zeta-potential.

#### 3.2. MIC, MBC, and FICI of the antimicrobials

The MIC, MBC, and FICI of LAE and sorbic acid individually and in combination against Salmonella Enteritidis ATCC H4267 and L. monocytogenes Scott A at pH 5.0, 6.0, and 7.0 are shown in Table 1. Both bacteria were more sensitive to LAE than sorbic acid. When LAE was tested individually, MIC values of  $\sim$ 45 ppm (0.0045% v/v) and  $\sim 25 \text{ ppm} (0.0025\% \text{ v/v})$  were observed at pH 5.0 and 6.0, respectively. In contrast, the MIC of sorbic acid was more strongly dependent on pH than LAE. At pH 5.0, the MIC of sorbic acid was detected at ca. 100 ppm  $(0.01\% \text{ w v}^{-1})$  against L. monocytogenes and 200 ppm (0.02% w v<sup>-1</sup>) against Salmonella Enteritidis; whereas, the MIC against both bacteria at pH 6.0 was 400 ppm (0.04% w v<sup>-1</sup>). After individual MICs were obtained, the antibacterial effect of the binary combination of the antimicrobials was evaluated. The analysis of FICI (Table 1) shows the additive effect (FICI = 1.0) at pH 5.0 and partially synergistic effect (FICI = 0.75) at pH 6.0 against Salmonella Enteritidis, while an indifferent effect (FICI = 1.25) was obtained for L. monocytogenes Scott A at both pH 5.0 and 6.0. The partial synergy against Salmonella at pH 6.0 and lower MIC than that at pH 7.0 led to the decision to adjust the coating pH to 6.0 for the rest of this study.

Antimicrobials must be active in food matrices with varied acidity. LAE, with reported stability at pH above 4.0,9 must also be stable in acidic conditions. The low MIC of LAE obtained in this study agrees with a previous study reporting MICs between 11 and 80 ppm against L. monocytogenes strains and from 20 to 250 ppm against different Salmonella strains in neutral growth media.18 In another study, Suksathit & Tangwatcharin25 reported MICs of 8 ppm and 16 ppm against L. monocytogenes and Salmonella Rissen, respectively. LAE interacts with lipids in bacterial membranes, disrupting membrane potential and causing structural changes in Gram-positive and Gram-negative bacteria.25 LAE can also cause aggregation of bacterial DNA molecules.16 In addition, LAE can induce expression of stressresponse genes in L. monocytogenes, indicating its impact on bacterial behaviour at the molecular level.26 Furthermore, the antimicrobial activity of LAE is thought to be directly associated

Table 1 Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and fractional inhibitory concentration index (FICI) of lauric arginate (LAE) and sorbic acid when tested alone or in combination against Salmonella Enteritidis ATCC H4267 and Listeria monocytogenes Scott A in tryptic soy broth adjusted to pH 5.0, 6.0, and 7.0

		LAE (ppm)		Sorbic acid (ppm)		Interaction	
Bacteria	pН	MIC* (alone/combined)	MBC	MIC* (alone/combined)	MBC	FICI	Interpretation
Salmonella enteritidis	5.0	45/33.75	50	200/50	400	1.0	Additive
	6.0	25/12.5	35	400/100	1500	0.75	Partially synergistic
	7.0	20/15	25	1500/750	4000	1.25	Indifferent
L. monocytogenes	5.0	45/33.75	50	100/50	400	1.25	Indifferent
	6.0	25/18.75	50	400/200	3000	1.25	Indifferent
	7.0	20/10	30	1500/750	4000	1.0	Additive

with its positive charge.27 The lower MIC of LAE at a higher pH (Table 1) does not seem to support this direct correlation because neutralization of the arginine guanidinium group by hydroxide ions at increased pH would reduce the positive charge and thus antimicrobial activity.

Similar MICs of 100-500 ppm against different microorganisms have been reported previously for sorbic acid when the media were adjusted to pH 5.0 or 6.0.28 The increased antimicrobial activity of sorbic acid at a lower pH is due to the higher concentration of undissociated acid molecules that are more hydrophobic than the salt form.29 The acid form in turn penetrates the cell membrane better to enter the bacteria to decrease the intracellular pH of microorganisms, thus disrupting catabolic pathways.30

The partial synergy observed at pH 6.0 against Salmonella (Table 1) can be attributed to the ability of LAE binding bacterial membranes, facilitating the penetration of sorbic acid.23 However, because sorbic acid itself is a small molecule and can effectively penetrate cell membranes,30 the enhancement by LAE may not be significant, corresponding to additive or indifferent effects at other conditions (Table 1). A synergistic effect between LAE and cinnamon essential oil against L. monocytogenes<sup>31</sup> and between LAE and peracetic acid against L. monocytogenes32 have been reported. The detailed mechanisms leading to different combination effects are to be studied in the future.

#### 3.3. Pathogen survival in the commercial coating dispersion

The pathogen survival in the coating with and without adjusting to pH 6.0 at RT is presented in Fig. 2. The commercial coating alone, at both pH levels, did not have any inhibitory effect against Salmonella Enteritidis ATCC H4267 and L. monocytogenes Scott A. For the coating without pH adjustment, no inhibition of either pathogen was observed at antimicrobial concentrations as high as 0.2% v/v LAE and 2.0% w per v sorbic acid.

For the coating adjusted to pH 6.0, a higher antimicrobial concentration led to lower populations of the bacteria, and the efficacy of the combined antimicrobials was significantly higher  $(p \le 0.05)$  against Salmonella Enteritidis than L. monocytogenes.

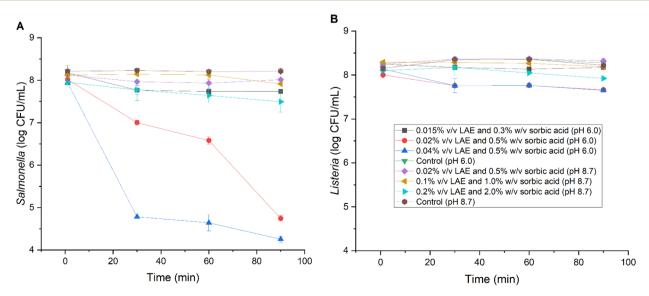


Fig. 2 Survival of Salmonella Enteritidis ATCC H4267 (A) or L. monocytogenes Scott A (B) in the coating without (pH 8.7) and with adjusting pH to 6.0 and supplementing with different concentrations of lauric arginate (LAE) and sorbic acid during incubation at 21 °C. Legends in (B) also apply for (A). Error bars represent SD (n = 2).

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At the highest antimicrobial concentrations tested (0.2% v/v LAE and 2.0% w per v sorbic acid), the reduction was 3.75 log CFU mL<sup>-1</sup> for Salmonella Enteritidis after 90 min, while it was only 0.48 log CFU mL<sup>-1</sup> for *L. monocytogenes*. Results in Fig. 2 proved the need of acidifying the coating to utilize the antibacterial activity of LAE and sorbic acid.

When comparing MIC and MBC in Table 1, the activity of LAE and sorbic acid combinations in the coating (Fig. 2) was much lower. This can be attributed to several factors. The coating has a negative zeta-potential (Fig. 1). The positively charged LAE therefore can bind with the negatively charged coating components. In addition, the coating had about 69% lipids. LAE is a surfactant and can bind with lipid bodies in the coating.9 Sorbic acid also can bind with lipid bodies in the coating via hydrophobic interactions, which can be less severe because sorbic acid can be applied in lipophilic environments.33 These binding mechanisms decrease the amount of antimicrobials available to interact with bacteria in the coating, lowering the measured activity. Furthermore, the antimicrobial activity difference of LAE and sorbic acid combinations against L. monocytogenes and Salmonella appears to be much greater in the coating (Fig. 2) than in the growth media (Table 1). In addition to the binding with the coating components making less LAE and sorbic acid available to inactivate bacteria, the coating components may support the survival of pathogens differently. Unfortunately, the coating composition is a trade secret. Further studies are needed to confirm the underlying mechanisms behind the combined effects of LAE and sorbic acid in the coating.

# 3.4. Survival of pathogens inoculated on fresh peaches after coating and storage

Fresh peaches used in this study did not harbor Salmonella and L. monocytogenes as confirmed by plating on selective media prior to experiments. The initial bacterial population in the peaches reported as day 0 was 5.7 and 6.4 log CFU per fruit for Salmonella and L.monocytogenes, respectively (Fig. 3). For Salmonella (Fig. 3A), the coated peaches had less than 0.29 log CFU per fruit reductions after 5 days of storage, and coating conditions did not result in significant differences in log reductions (p > 0.05). After 10 days, the high-level antimicrobial treatment reduced the Salmonella count by about 1.58  $\pm$  0.28 log CFU per fruit compared to just 0.46  $\pm$  0.24 log CFU per fruit in the coating control without antimicrobials. After 20 days of storage, the high-level antimicrobial treatment achieved 2.17  $\pm$ 0.50 log CFU per fruit reduction of Salmonella, significantly (p < 0.05) higher than the low level (1.20  $\pm$  0.68 log CFU per fruit reduction) and no (0.41  $\pm$  0.51 log CFU per fruit reduction) antimicrobial treatments. In contrast, the uncoated control peaches also showed gradual reductions of Salmonella during storage, and the reduction at the end of storage (2.07  $\pm$  0.49 log CFU per fruit) was similar (p > 0.05) to the high-level antimicrobial treatment.

The L. monocytogenes populations among the coating conditions were not significantly different (p > 0.05) on the same day and were significantly reduced ( $p \le 0.05$ ) during storage at 0 °C (Fig. 3B). For the uncoated fruits, the highest reduction of *L. monocytogenes* population by  $2.90 \pm 0.82 \log CFU$  per fruit was observed after 5 days ( $p \le 0.05$ ), followed by another reduction on day 10 ( $p \le 0.05$ ) and no significant difference thereafter (p >0.05). Overall, the coating treatments with and without antimicrobial had higher L. monocytogenes populations than the uncoated control.

For the peaches stored at 21 °C, the high-level antimicrobial treatment reduced the Salmonella count by 1.49  $\pm$  0.63 log CFU per fruit after 5 days, similar (p > 0.05) to the uncoated control peaches (Fig. 4A). The coating conditions did not affect the L. monocytogenes population throughout storage (p > 0.05). Moreover, the L. monocytogenes count significantly decreased ( $p \le$ 0.05) in peaches coated with the high-level antimicrobial after 5 days at 21 °C (Fig. 4B).

Furthermore, the population of L. monocytogenes was lower than that of Salmonella after the same storage time at 0 °C. Several factors impact the results in Fig. 3. L. monocytogenes as an opportunistic bacterium survives better at temperatures (4 ° C) cooler than those in crop production environments of 15-20 °C.34 Even if cold environments decrease the bacterial growth

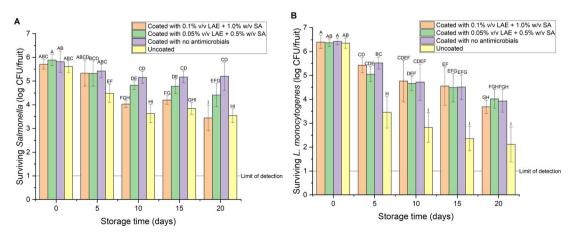


Fig. 3 Survival of Salmonella (A) and Listeria monocytogenes (B) cocktails inoculated on peaches without and with spraying with the coating adjusted to pH 6.0 and supplemented with different concentrations of lauric arginate (LAE) and sorbic acid (SA) during 20 days storage at 0 °C and 85% relative humidity. Error bars represent SD (n = 12). Different uppercase letters denote significant differences (p < 0.05) among all data points.

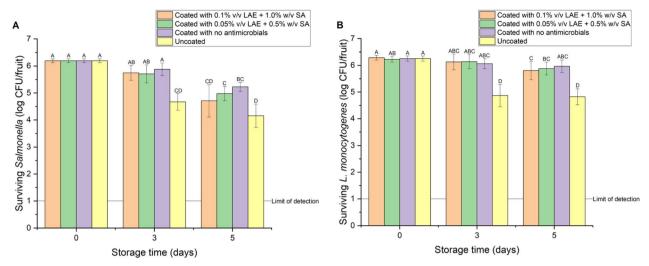


Fig. 4 Survival of Salmonella (A) and Listeria monocytogenes (B) cocktails inoculated on peaches without and with spraying with the coating adjusted to pH 6.0 and supplemented with different concentrations of lauric arginate (LAE) and sorbic acid (SA) during 5 days storage at 21 °C and 85% relative humidity. Error bars represent SD (n = 12). Different uppercase letters denote significant differences (p < 0.05) among all data points.

rate, L. monocytogenes can grow at temperatures as low as  $-0.4^{\circ}$ C and survive in freezing temperatures such as −18 °C.35 Salmonella can also survive at 0 °C and the decline in its population during cold storage is common.<sup>36</sup> No apparent reduction of L. monocytogenes on peaches was previously reported after applying commercial stone fruit coatings from Pace International, LLC (based on mineral oil or vegetable oil with fungicide propiconazole or fludioxonil) and storing at 1-2 °C and 85-95% RH for 28 days.21 The results in Fig. 3 for uncoated peaches agree with another study showing about 1 log reduction of L. monocytogenes inoculated on nectarines after storage at 2  $^{\circ}$ 

C for 2 days and 22 °C for 2 additional days.37 However, these two studies did not report how peaches were packed, which may cause the difference between their and our studies. In our study, peaches were stored in open trays, and dehydration in the storage room may lower the water activity on peach surface to cause the reduction of inoculated pathogens from desiccation stress. In addition to preserving water activity, the better survival of both pathogens in the coating control (without antimicrobial) treatment than the uncoated control may suggest the protection of the coating on pathogen survival storage. 21,37 Additional reductions in Salmonella

Table 2 Total soluble solids (TSS), titratable acidity (TA, malic acid% equivalent), and weight loss of peaches without and with spraying with the coating adjusted to pH 6.0 and supplemented with no, low level (0.05% v/v lauric arginate and 0.5% w per v sorbic acid), and high level (0.1% v/v lauric arginate and 1.0% w per v sorbic acid) antimicrobials during 20 days storage at 0 °C and 85% relative humidity

Coating conditions	Storage time (days)	TSS (°Brix)	TA (%)	рН	Weight loss (%)
Coating with high level antimicrobial	0	$11.5^{\rm c}\pm0.5$	$7.0^{\mathrm{a,b,c,d}} \pm 1.5$	$3.8^{ m d,e,f} \pm 0.1$	_
	5	$11.8^{\rm c}\pm1.5$	$8.2^{\mathrm{a,b}}\pm1.9$	$3.7^{\rm f}\pm0.1$	$2.4^{\rm h}\pm0.4$
	10	$12.1^\mathrm{b,c}\pm1.4$	$7.2^{a,b,c,d} \pm 1.2$	$3.8^{\mathrm{d,e,f}}\pm0.1$	$4.1^{\rm h}\pm0.6$
	15	$12.6^{\mathrm{b,c}}\pm1.1$	$5.9^{\mathrm{a,b,c,d}}\pm1.4$	$4.3^{\mathrm{a,b}}\pm0.2$	$8.3^{\rm e,f}\pm1.1$
	20	$13.5^{ m b,c} \pm 1.0$	$5.3^{ m b,c,d} \pm 1.4$	$3.8^{ m e,f} \pm 0.3$	$11.6^{\rm c}\pm1.2$
Coating with low level antimicrobial	0	$11.5^{\rm c}\pm0.5$	$7.0^{\mathrm{a,b,c,d}} \pm 1.5$	$3.8^{\mathrm{d,e,f}}\pm0.1$	_
	5	$12.0^\mathrm{b,c}\pm0.2$	$7.5^{a,b,c} \pm 1.6$	$3.9^{\mathrm{d,e,f}}\pm0.1$	$2.7^{\rm h}\pm0.4$
	10	$12.8^{ m b,c}\pm 1.6$	$6.9^{a,b,c,d} \pm 1.3$	$4.0^{\mathrm{c,d,e,f}} \pm 0.1$	$4.6^{ m g,h}\pm0.8$
	15	$11.9^{\rm c}\pm1.1$	$5.5^{ m a,b,c,d} \pm 0.8$	$4.3^a \pm 0.1$	$8.8^{ m d,e}\pm1.5$
	20	$12.8^\mathrm{b,c}\pm1.1$	$5.9^{ m a,b,c,d} \pm 1.2$	$3.9^{\mathrm{c,d,e,f}} \pm 0.2$	$12.1^{\mathrm{c,d}} \pm 2.1$
Coating with no antimicrobials	0	$11.5^{\rm c}\pm0.5$	$7.0^{\mathrm{a,b,c,d}} \pm 1.5$	$3.8^{\mathrm{d,e,f}}\pm0.1$	_
	5	$11.6^{\rm c}\pm0.9$	$7.8^{ m a,b,c} \pm 1.1$	$3.9^{{ m c,d,e,f}}\pm 0.1$	$2.3^{\rm h}\pm0.1$
	10	$11.5^{\rm c}\pm0.6$	$7.8^{a,b,c} \pm 1.6$	$4.0^{\mathrm{c,d,e,f}} \pm 0.1$	$4.1^{\rm h}\pm0.3$
	15	$13.2^{ m b,c}\pm 1.1$	$4.2^{ m d}\pm0.7$	$4.4^{\rm a}\pm0.1$	$8.6^{ m e,f}\pm0.5$
	20	$12.3^{\mathrm{b,c}}\pm0.4$	$5.1^{\mathrm{c,d}}\pm0.5$	$4.0^{\rm b,c,d}\pm0.1$	$12.2^{\mathrm{c,d}}\pm0.7$
Uncoated	0	$11.5^{\rm c}\pm0.5$	$7.0^{ m a,b,c,d}\pm 1.5$	$3.8^{\mathrm{d,e,f}}\pm0.1$	_
	5	$12.1^{ m b,c}\pm 1.2$	$8.3^{a} \pm 0.8$	$3.8^{\mathrm{d,e,f}}\pm0.1$	$8.0^{\rm f,g}\pm1.1$
	10	$13.5^{\mathrm{b,c}} \pm 0.7$	$6.9^{a,b,c,d} \pm 1.6$	$4.0^{\mathrm{b,c,d,e}}\pm0.2$	$14.8^{\rm c}\pm1.8$
	15	$14.1^{ m a,b}\pm 1.7$	$7.8^{\mathrm{a,b,c}} \pm 2.5$	$4.2^{\mathrm{a,b,c}}\pm0.1$	$30.2^{\mathrm{b}} \pm 3.7$
	20	$16.2^a\pm1.4$	$5.5^{ m a,b,c,d} \pm 0.9$	$4.2^{\mathrm{a,b,c}}\pm0.1$	$40.7^{\rm a}\pm4.1$

<sup>&</sup>lt;sup>a</sup> Numbers are mean  $\pm$  SD (n=6), Mean values in the same column with different superscript letters differ significantly (p < 0.05).

populations for the treatments with LAE and sorbic acid at both storage temperatures (Fig. 3 and 4) when compared to the coating control show the net effect of the antimicrobials. However, further optimization of coating formulation or combination with other interventions may be necessary to achieve higher log reductions suitable for commercial application.

It is well known that the *in vitro* activity of antimicrobials in simple media is lower than the *in vivo* activity, when applied in or on food matrices.<sup>38</sup> In the present study, the reduction of pathogens on peaches (Fig. 3 and 4) is less than that in the coating (Fig. 2), even after a longer period. The lower temperature of peaches may not be a factor because a lower MIC was observed at a lower temperature when *L. monocytogenes* was tested at 4, 10, and 37 °C and *Salmonella* at 10, 25, and 37 °C.<sup>39</sup> As discussed previously for the binding between the antimicrobials and the coating components, the same physical phenomena can occur between the antimicrobials and the peach components, such as fat, polysaccharide, and protein.<sup>18</sup>

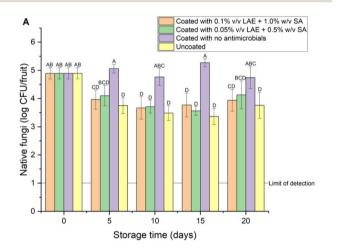
The peach surface has two types of hydrophobic structures: a trichome layer and the cuticle that are made of different proportions of waxes of different polarity, as well as cutan in the cuticle. However, brushing peaches before applying the coating can additionally expose non-lipid components to bind the antimicrobials, making them less available to reach the inoculated pathogens. Additionally, drying the coating on peaches creates a hydrophobic environment and the reduced water activity, both of which could trigger *Listeria* and *Salmonella* to adapt to these adverse environmental conditions to help their survival during storage. This may explain the lower population of pathogens on the uncoated peaches than the coating only control (Fig. 3 and 4). Further research is needed to understand the survival of bacteria on peaches under the conditions studied.

Results in Fig. 3 indicate the greater efficacy of LAE and sorbic acid against L. monocytogenes than Salmonella inoculated on peaches, which disagrees with the results in the coating (Fig. 2). Salmonella can grow at pH as low as 3.9 when the water activity is greater than 0.96, while L. monocytogenes does not grow below pH 4.2 regardless of the water activity.42 In the present study, peaches were brushed, and the pathogens inoculated on peaches may be exposed to peach flesh. As presented below, the peach flesh pH was 3.8 (Table 2), and this low pH may have caused the higher reduction of L. monocytogenes than Salmonella and have led to the insignificant effect of LAE and sorbic acid in the coating against L. monocytogenes (Fig. 3). In addition, malic acid is the predominant organic acid in peaches and may have further enhanced L. monocytogenes inactivation at low pH.43 Nevertheless, the ability of both pathogens to survive on coated and uncoated peaches suggests the need for stone fruit packinghouses to implement preventive controls.<sup>37</sup>

# 3.5. Survival of native fungi on coated or uncoated peaches during storage

As presented in Fig. 5A, the coating control (without antimicrobial) treatment showed insignificant changes (p > 0.05) in

native fungi during storage at 0 °C. While for other treatments, a significant reduction (p < 0.05) was observed after 5 days, followed by insignificant changes after storage for longer times (p > 0.05). There was also no significant difference between the uncoated control peaches and those coated with antimicrobials (p > 0.05). When stored at 21 °C, coated peaches without antimicrobials had a significantly higher native fungi count compared to those coated with the high-level antimicrobial and the uncoated control after 5 days (p < 0.05) (Fig. 5B). These results suggest that the commercial coating itself may promote the survival of native fungi, and the antimicrobials studied when supplemented in the coating can be used to inhibit the fungi. It should be noted that fungicides such as propiconazole and fludioxonil are used in the packinghouse.21 However, these fungicides are not as friendly as GRAS food preservatives. Identifying native fungi and the impact of coating treatments will be a significant future research topic.



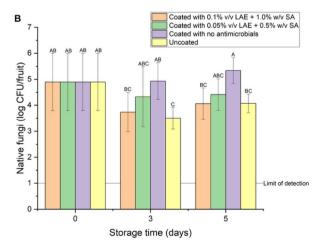


Fig. 5 Native fungi survival in uninoculated peaches without and with spraying with the coating adjusted to pH 6.0 and supplemented with different concentrations of lauric arginate (LAE) and sorbic acid (SA) during 20 days storage at 0 °C (A) or 5 days storage at 21 °C (B) and 85% relative humidity. Error bars represent SD (n = 12). Different uppercase letters denote significant (p < 0.05) differences among all data points.

# 3.6. Quality parameters of coated or uncoated peaches during storage

As summarized in Table 2, the TSS in coated or uncoated peaches during 20 days storage ranged from 11.5  $\pm$  0.5 to 16.2  $\pm$ 1.4 °Brix. There was no significant change in the TSS of peaches applied with the coating during storage (p > 0.05), and there was no significant difference among the coating treatments (p > p)0.05). In contrast, the TSS of the uncoated peaches increased significantly (p < 0.05) during storage and was significantly higher than the coated peaches at the end of storage. A similar trend was observed for uncoated peaches stored at 21 °C, where

the TSS reached 17.3  $\pm$  1.8 °Brix after 5 days (Table 3). TA values of 4.2  $\pm$  0.7% to 8.3  $\pm$  0.8% at 0 °C (Table 2) and 5.8  $\pm$  1.2% to  $8.3 \pm 2.0\%$  at 21 °C (Table 3) were similar among treatments and throughout storage (p > 0.05). pH values ranged from 3.7  $\pm$ 0.1 to 4.4  $\pm$  0.1, and there was no apparent change during storage at 0 °C (p > 0.05) for the coated peaches and no apparent impact of the coating composition (p > 0.05) (Table 2). For the uncoated peaches, pH increased during storage, being significantly higher on day 15 and 20 (p < 0.05). For the peaches stored at 21 °C, the pH was significantly higher after 5 days (p < 0.05) when coated with the high-level antimicrobial (Table 3). For the

Table 3 Total soluble solids (TSS), titratable acidity (TA, malic acid% equivalent), and weight loss of peaches without and with spraying with the coating adjusted to pH 6.0 and supplemented with no, low level (0.05% v/v lauric arginate and 0.5% w per v sorbic acid), and high level (0.1% v/v lauric arginate and 1.0% w per v sorbic acid) antimicrobials during 5 days storage at 21 °C and 85% relative humidity<sup>a</sup>

Coating conditions	Storage time (days)	TSS (°Brix)	TA (%)	pH	Weight loss (%)
Coating with high level antimicrobial	0	$11.5^{\rm c} \pm 0.5$	$7.0^{a} \pm 1.5$	$3.8^{\mathrm{b}}\pm0.1$	_
0 0	3	$11.8^{\rm c}\pm0.4$	$6.1^{\rm a}\pm1.0$	$4.1^{\rm a}\pm0.1$	$9.5^{\mathrm{e}}\pm1.1$
	5	$14.0^{\mathrm{b}}\pm1.3$	$6.1^{\mathrm{a}}\pm1.9$	$4.2^a\pm0.3$	$15.7^{\rm d}\pm1.6$
Coating with low level antimicrobial	0	$11.5^{\rm c}\pm0.5$	$7.0^{a} \pm 1.5$	$3.8^{\rm b}\pm0.1$	_
	3	$12.4^\mathrm{b,c}\pm1.0$	$5.8^{\mathrm{a}}\pm1.2$	$4.2^a\pm0.1$	$11.6^{\rm e}\pm2.0$
	5	$12.7^\mathrm{b,c}\pm0.6$	$8.2^{\mathrm{a}}\pm1.6$	$4.0^{\mathrm{a,b}}\pm0.1$	$18.9^{ m b,c}\pm 3.2$
Coating with no antimicrobials	0	$11.5^{\rm c}\pm0.5$	$7.0^{a} \pm 1.5$	$3.8^{\mathrm{b}}\pm0.1$	_
	3	$12.7^\mathrm{b,c}\pm1.0$	$7.0^{a} \pm 1.3$	$4.2^{\rm a}\pm0.2$	$9.5^{\rm e} \pm 0.9$
	5	$13.2^{ m b,c} \pm 0.6$	$7.2^{\mathrm{a}}\pm1.2$	$4.0^{\mathrm{a,b}}\pm0.1$	$15.3^{ m d} \pm 0.6$
Uncoated	0	$11.5^{\rm c}\pm0.5$	$7.0^a \pm 1.5$	$3.8^{\rm b}\pm0.1$	_
	3	$14.0^{\rm b}\pm1.1$	$6.5^{\mathrm{a}}\pm1.5$	$4.2^{\rm a}\pm0.1$	$19.7^{\mathrm{b}}\pm2.4$
	5	$17.3^a\pm1.8$	$8.3^a\pm2.0$	$4.1^{\mathrm{a,b}}\pm0.1$	$31.1^a\pm3.0$

<sup>&</sup>lt;sup>a</sup> Numbers are mean  $\pm$  SD (n = 6). Mean values in the same column with different superscript letters differ significantly (p < 0.05).

Table 4 Colour parameters of peaches without and with spraying with the coating adjusted to pH 6.0 and supplemented with no, low level (0.05% v/v lauric arginate and 0.5% w per v sorbic acid), and high level (0.1% v/v lauric arginate and 1.0% w per v sorbic acid) antimicrobials during 20 days storage at 0 °C and 85% relative humidity<sup>a</sup>

		Colour parameters			
Coating conditions	Storage time (days)	$L^*$	a*	<i>b</i> *	
Coating with high level antimicrobial	0	$62.9^{a} \pm 7.7$	$25.1^a \pm 7.5$	$47.5^{a} \pm 8.3$	
	5	$59.6^a\pm12.0$	$26.3^a \pm 9.5$	$44.6^a\pm10.7$	
	10	$61.6^{\rm a}\pm13.1$	$24.4^{\rm a}\pm7.2$	$45.8^a\pm13.3$	
	15	$60.1^{\mathrm{a}}\pm11.8$	$25.3^{a} \pm 8.6$	$42.9^a\pm11.9$	
	20	$61.3^{\mathrm{a}}\pm13.4$	$23.4^{\rm a}\pm 9.4$	$46.0^a\pm12.3$	
Coating with low level antimicrobial	0	$63.8^{\mathrm{a}}\pm9.1$	$22.6^{\rm a}\pm9.9$	$45.9^{a} \pm 8.3$	
	5	$63.4^{\mathrm{a}}\pm12.1$	$23.0^a \pm 9.8$	$48.0^a\pm12.0$	
	10	$62.7^{ m a}\pm 12.3$	$25.3^{ m a} \pm 7.1$	$46.9^a\pm10.4$	
	15	$60.4^{\mathrm{a}} \pm 13.0$	$25.6^a\pm 9.2$	$44.5^a\pm12.1$	
	20	$56.3^{a} \pm 13.8$	$26.9^{a} \pm 7.9$	$41.3^a\pm13.7$	
Coating with no antimicrobials	0	$60.5^{\rm a}\pm 9.2$	$19.9^{\mathrm{a}}\pm8.0$	$45.7^{\mathrm{a}}\pm8.1$	
	5	$64.4^{\rm a} \pm 9.50$	$22.0^{a} \pm 7.8$	$50.0^{\rm a}\pm9.0$	
	10	$64.0^{\mathrm{a}}\pm13.0$	$23.7^{\mathrm{a}} \pm 7.9$	$47.6^a\pm13.0$	
	15	$64.5^{\mathrm{a}}\pm11.4$	$23.9^{a} \pm 10.6$	$49.3^a\pm11.1$	
	20	$58.0^{\mathrm{a}} \pm 15.1$	$24.6^{\rm a}\pm 9.5$	$42.5^a\pm15.6$	
Uncoated	0	$61.5^{\rm a}\pm8.1$	$20.6^{a} \pm 7.8$	$42.7^a \pm 9.1$	
	5	$60.6^{\mathrm{a}} \pm 12.5$	$26.1^{a} \pm 8.3$	$42.8^a\pm14.4$	
	10	$65.5^{\mathrm{a}}\pm11.0$	$23.8^a\pm7.9$	$44.9^a\pm11.6$	
	15	$64.8^{\mathrm{a}}\pm10.4$	$23.1^{\rm a}\pm6.6$	$45.7^a\pm10.9$	
	20	$64.4^{\mathrm{a}}\pm11.1$	$21.5^{\rm a}\pm7.0$	$43.7^a\pm12.2$	

<sup>&</sup>lt;sup>a</sup> Values are mean  $\pm$  SD (n = 6). Shared superscript letters in the same column do not show significant differences (p > 0.05).

Table 5 Colour parameters of peaches without and with spraying with the coating adjusted to pH 6.0 and supplemented with no, low level (0.05% v/v lauric arginate and 0.5% w per v sorbic acid), and high level (0.1% v/v lauric arginate and 1.0% w per v sorbic acid) antimicrobials during 5 days storage at 21 °C and 85% relative humidity<sup>a</sup>

		Color parameters			
Coating conditions	Storage time (days)	$L^*$	a*	<i>b</i> *	
Coating with high level antimicrobial	0	$62.9^{a}\pm7.7$	$25.1^a \pm 7.5$	$47.5^a\pm8.3$	
	3	$61.4^{a} \pm 9.5$	$27.7^{a} \pm 8.0$	$46.3^a\pm10.8$	
	5	$58.5^{a} \pm 9.5$	$27.7^{a} \pm 6.9$	$45.8^a\pm11.0$	
Coating with low level antimicrobial	0	$63.8^{a} \pm 9.1$	$22.6^{\rm a} \pm 9.9$	$45.9^{\mathrm{a}}\pm8.3$	
	3	$60.0^{a} \pm 13.7$	$23.9^{\rm a}\pm4.4$	$46.4^a\pm15.5$	
	5	$61.4^{\rm a} \pm 8.5$	$23.3^{\rm a} \pm 6.4$	$50.2^a\pm09.1$	
Coating with no antimicrobials	0	$60.5^{\mathrm{a}} \pm 9.2$	$19.9^{a} \pm 8.0$	$45.7^{\mathrm{a}} \pm 8.1$	
	3	$58.2^a\pm11.2$	$27.0^{\mathrm{a}}\pm8.7$	$44.4^a\pm13.1$	
	5	$61.7^{\mathrm{a}} \pm 7.4$	$25.7^{\rm a} \pm 6.3$	$48.4^a\pm10.4$	
Uncoated	0	$61.5^{\mathrm{a}} \pm 8.1$	$20.6^{\rm a} \pm 7.8$	$42.7^{\mathrm{a}} \pm 9.1$	
	3	$64.1^a \pm 9.2$	$25.3^{ m a} \pm 5.7$	$47.1^a\pm10.7$	
	5	$61.6^{a} \pm 6.4$	$26.3^a\pm4.8$	$43.6^a\pm8.6$	

<sup>&</sup>lt;sup>a</sup> Values are mean  $\pm$  SD (n = 6). Shared superscript letters in the same column do not show significant differences (p > 0.05).

weight loss, a longer storage time led to a more significant impact (p < 0.05), and the coated peaches had a significantly lower weight loss than the uncoated peaches (p < 0.05) after the same storage time. After 10 days of storage, the uncoated peaches lost more than twice the amount of weight as the coated peaches, and this difference was more than three times after 20 days of storage at 0 °C (Table 2). Overall, the addition of antimicrobials had an insignificant impact on the weight loss of peaches (p > 0.05). Moreover, storing the peaches at 21 °C resulted in significant weight loss after 5 days, with the highest value obtained for the uncoated peaches (Table 3). When color parameters were compared in Tables 4 and 5, there was no significant difference among the treatments throughout storage at both temperatures.

Since fresh fruits are susceptible to excessive weight loss and quality deterioration, edible coatings are used to control moisture transfer and gas exchange and improve appearance and quality during long storage.5 Our results agree with some studies. Alegre et al.44 reported pH values of 3.49-4.73, TSS between 8.9 and 14.7, and TA values of 4.1-8.6% malic acid equivalent from various varieties of peaches. Hazrati et al. 45 mentioned that the increasing TSS during storage was due to starch metabolism and its conversion to sugar and other soluble solids, but the same study reported TA decreasing by 30% during the ripening process of peach. In the present study, biochemical reactions at 0 °C are expected to be inactive, and the higher TSS of uncoated than coated peaches can be simply due to the inability to prevent moisture loss during storage (high weight loss; Table 2). Results in Table 2 agree with a previous study reporting more than 35% weight loss in uncoated peaches after 26 days of storage at 4 °C,46 but the weight loss of coated peaches in this study was less severe than up to 15% for peaches treated with alginate-based coating and stored for 20 days at 15 °C.47 Overall, results in Tables 2-5 suggest the function of the commercial coating in maintaining the quality of stone fruits and no negative impacts of the

studied antimicrobials in compromising this function. Future research on sensory properties of peaches as affected by the coating and the antimicrobials, as well as large scale decay characterization, will be needed for practical applications.

# 4 Conclusions

This study showed that supplementing a commercial stone fruit coating with LAE and sorbic acid can enhance its antimicrobial activity. Reducing the alkaline coating's pH to 6.0 resulted in the inactivation of Salmonella and L. monocytogenes by LAE and sorbic acid, with a greater impact on Salmonella. When the pHadjusted coating was supplemented with LAE and sorbic acid and applied to peaches, a more significant reduction of L. monocytogenes than Salmonella was observed during storage at 0 °C for up to 20 days, likely due to the peach pH effect. However, both bacteria survived on the peaches during cold storage. In addition, the coating itself seemed to have protected L. monocytogenes during storage both at 0 °C and 21 °C. The incorporation of LAE and sorbic acid in the coating reduced the survival of native fungi on the peaches and did not have any impact on the quality of the coated peaches. Despite the need to further improve the log reductions of pathogens and understand the pathogen survival on fresh peaches, the development of safe and effective antimicrobial edible coatings is a promising area for future research that could leverage existing production methods to improve the microbial safety and sustainable production of fresh peaches.

## Author contributions

Fatima Reyes-Jurado: conceptualization, methodology, formal analysis, investigation, writing – original draft, visualization. Kriza Faye Calumba: conceptualization, methodology, formal analysis, investigation, writing – original draft, visualization. Thomas G. Denes: methodology, resources, writing – review & editing, funding acquisition. Qixin Zhong: conceptualization,

methodology, resources, writing - review & editing, supervision, project administration, funding acquisition.

# Conflicts of interest

There are no conflicts to declare.

# Data availability

Supplementary information: The data supporting this article have been included as part of the SI. See DOI: https://doi.org/ 10.1039/d5fb00434a.

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