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Survival of Salmonella Typhimurium and Escherichia coli O157: H7 on blueberries and impacts on berry quality during 12 weeks of frozen storage after washing with combinations of sodium dodecyl sulfate and organic acids or hydrogen peroxide

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Abstract

Salmonella spp. and Escherichia coli are well tolerant of freezing. This study was to investigate survival of the foodborne pathogens during storage at −18 ± 2°C for 12 weeks on blueberries after washing with: 500 ppm acetic acid plus 5,000 ppm sodium dodecyl sulfate (SDS) (AA/SDS), 20 ppm peroxyacetic acid plus 5,000 ppm SDS (PPA/SDS), or 200 ppm hydrogen peroxide plus 5,000 ppm SDS (H_2O_2/SDS), when compared with findings from no wash, or wash with water, 80 ppm PPA or 200 ppm chlorinated water. Following a 60 s contact with one of the three new solutions, the treatments showed 3.3–3.9 $\log_{10}$ CFU/g reductions in Salmonella Typ-himurium and E. coli O157:H7 counts. After 2 weeks of frozen storage, 3.9–4.2 $\log_{10}$ CFU/g reductions of Salmonella and E. coli were observed. After 12 weeks of frozen storage, Salmonella and E. coli survivors were below detection limits (0.39 $\log_{10}$ CFU/g) in berries washed with new solutions. The frozen storage had a significant impact ($p < .05$) on microbial counts of both treated and nontreated blueberries. Although none of these washings decreased the total phenolic and anthocyanins contents and apparent quality at time 0, frozen storage caused significant damage on the texture of both treated and nontreated blueberries. Interestingly, no significant decrease in the total phenolic, anthocyanins content, and apparent quality was observed during the 12-week frozen storage. The counts of total bacteria, yeasts, and molds decreased throughout storage for treated and untreated berries. This dem-onstrates that the three wash solutions enhance the safety of frozen berries.

1 INTRODUCTION

Freezing is the most traditional method of preserving berries since it provides a long shelf life and minimal impacts on quality and nutrition. Although illnesses associated with frozen berries are rare, freezing also preserves the viability of some pathogenic microorganisms. According to center for disease control (CDC), there has not been any *Escherichia coli* O157:H7 outbreak related with blueberries; however, pathogenic bacteria such as *Salmonella* and viruses such as hepatitis A virus that are often transmitted via the fecal-oral route have all been linked to contaminated blueberries (CDC, 2013), which suggests that *E. coli* O157:H7 may also become potential contamination source for blueberries because this pathogen also causes contamination via fecal-oral route.

Berries picked for frozen processing are usually full-flavored, ripe berries with uniform size and tender skins. The berries are usually

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washed or sprayed with chlorinated water containing 50–200 ppm active chlorine to reduce microorganisms (good agricultural and manufacturing [handling] safety and food defense practices; North American Blueberry Council, 2010); however, this chlorine-based washing solution has shown limited efficacy to inactivate foodborne pathogens on blueberries, with 0.83, 0.77, and 0.61 $\log_{10}$ CFU/g reductions of bacteria, yeast, and mold after washing with 100 ppm chlorine for 5 min (Crowe, Bushway, & Bushway, 2005). The chlorine-based washing solutions also bring increased scrutiny from regulatory agencies, for example, the ban of chlorinated water washing on fresh produce in some European countries such as Germany and Denmark due to the undesirable chlorine by-products (Artes, Gomez, Aguayo, Escalona, & Artes-Hernundez, 2009; Nieuwenhuijsen, Toledano, & Elliot, 2000; Rico, Martin-Diana, Barat, & Barry-Ryan, 2007). Hence, because of limited effectiveness and issues surrounding public health and environmental safety, there is a need for more effective and practical methods for removal of pathogens from fresh blueberries prior to freezing.

Sodium dodecyl sulfate (SDS) is a surface-active compound that lowers the interfacial tension between two liquids or between a liquid and a solid. SDS is an U.S. food and drug administration (FDA)-approved multipurpose food additive (FDA, 2021) for use as emulsifier (≤1,000 ppm for egg white solids; 125 ppm for frozen and liquid egg white), whipping agent (≤0.5% [5,000 ppm]) by weight of gelatine in the preparation of marshmallows), or surfactant (≤25 ppm in beverage and fruit juice). In our study, SDS was tested based on the allowable levels when used as whipping agent. Addition of SDS to a levulinic-acid solution enhanced removal of *Salmonella* on chicken breast meats (Zhao, Zhao, & Doyle, 2009), and combinations of SDS with chlorinated water improved inactivation of human norovirus surrogates on produce (Predmore & Li, 2011). Enhanced inactivation of *Salmonella* Typhimurium on blueberries was determined after washing with solutions combining SDS (50, 500, and 5,000 ppm) with other antimicrobials (lactic acid, acetic acid, citric acid, or hydrogen peroxide) (Li & Wu, 2013). Three washing solutions, 500 ppm acetic acid plus 5,000 ppm SDS (AA/SDS), 20 ppm peroxyacetic acid combined with 5,000 ppm SDS (PPA/SDS), or 200 ppm hydrogen peroxide containing 5,000 ppm SDS (H_2O_2/SDS), resulted in more than 3 $\log_{10}$ CFU/g reductions of *Salmonella* Typhimurium. To further evaluate the antimicrobial efficacy of the three effective washing treatments, the one objective of this study was to evaluate their impacts on *E. coli* O157:H7 surface inoculated on blueberries. Additional objective was to determine the survival of *Salmonella* and *E. coli* on the treated blueberries stored in −18 ± 2°C for 12 weeks. Last objective of our study was to test the impacts of these treatments on sensory quality, total phenolic, anthocyanins content, molds and yeasts, and total bacteria accounts during the frozen storage of the blueberries.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains and inoculum preparation

Salmonella enterica serovar Typhimurium (DT 104) and *E. coli* O157:H7 strain 250 (sprout outbreak isolate) obtained from the culture collection in the Department of Animal and Food Sciences at the University of Delaware (Newark, DE). A mutant of the *E. coli* strain was isolated that is able to grow in the presence 100 μg/ml of nalidixic acid (Fisher Scientific, Hampton, NH) and 100 μg/ml of streptomycin (Sigma-Aldrich, St. Louis, MO). Stock cultures of *Salmonella* on tryptic soy agar (TSA; Difco Laboratories, Becton Dickinson, Spark, MD) and *E. coli* on TSA plus 0.6% yeast extract (Difco) supplemented with 100 μg/ml of nalidixic acid and 100 μg/ml of streptomycin (TSAYE-NS) were stored at 4°C. A single colony of *Salmonella* and *E. coli* from TSA plate was cultured into tryptic soy broth (TSB, Difco) and TSB plus 0.6% yeast extract (Fisher) supplemented with same antibiotics (TSBYE-NS), respectively, and then grown at 37°C for 24 hr. The culture was transferred to fresh TSB and TSBYE-NS and incubated at 37°C for another 24 hr. Before inoculation on the blueberries, viable counts were determined by serially diluting suspensions in sterile 0.1% peptone water (Difco) and spread plating 0.1 ml on the xylose lysine deoxycholate (XLD; Difco) plate for *Salmonella* and TSAYE-NS for *E. coli*.

2.2 | Contamination of blueberries surface

Fresh blueberries were obtained from a local market on the day of experimentation. Intact surfaces were selected for inoculation. Inoculation was achieved by applying 25 μl of inoculum (6–10 small droplets) onto the surface of each blueberry. After inoculation, blueberries were left in a laminar flow hood at room temperature (21 ± 1°C) for about 2 hr to allow for attachment of the microorganisms. Inoculated blueberries had approximately 10^5 CFU/g of *Salmonella* and *E. coli*.

2.3 | Washing procedures and frozen storage

The washing solutions included: 200 ppm free chlorine (prepared from sodium hypochlorite, Sigma–Aldrich, determined by Hach Company Free Chlorine Test Strips), 80 ppm peracetic acid (the concentration calculated and prepared from 32% peracetic acid, Sigma–Aldrich), combinations of 5,000 ppm SDS (Sigma–Aldrich, 99.0%) with one of the following antimicrobials: acetic acid (500 ppm, prepared from 99.7% acetic acid, Sigma–Aldrich), peracetic acid (20 ppm, the concentration calculated and prepared from 32% peracetic acid, Sigma–Aldrich), or hydrogen peroxide (200 ppm, prepared from 30% hydrogen peroxide, Sigma–Aldrich). Distilled water (DI) washing served as negative control. Blueberries at 50 g were submerged in 1,000 ml of the washing solutions with continuous agitation provided by stirring bar in a beaker for 1 min. For further evaluation of the antimicrobial efficacy of these effective treatments and their impacts on blueberry qualities, as described in Sections 2.6 and 2.7, blueberries of 80 g were used.

Following each treatment, samples were removed for microbial analysis at time 0 and transferred to commercially plastic storage bags prior to storage at −18 ± 2°C for 12 weeks. Viable cells of *Salmonella* and *E. coli* after each treatment were determined at 1, 2, 4, 8, and 12 weeks.

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2.4 | Microbial analysis

After washing, two randomly picked blueberries (approximately 4 g) from each treatment were placed in a sterile stomacher sample bag containing 10 ml of elution buffer (phosphate-buffered saline [PBS]) and pummeled in a stomacher (Colworth Stomacher 400, A. J. Seward and Co., Ltd., London, UK) for 2 min at medium speed. For the quantification of surviving bacteria, 1 ml aliquots from 10 ml of homogenate were plated on three XLD agar plates for *Salmonella* and three TSAYE-NS plates for *E. coli*. Plates were incubated at 37°C for 24 hr before presumptive-positive colonies were counted (Andrews, Jacobson, & Hammack, 2011). The microbial population detection limit was 0.39 $\log_{10}$ CFU/g.

Salmonella enrichment was carried out by adding 10 ml of TSB to the PBS homogenate and incubating at 37°C for 24 hr. After incubation, a loopful of enrichment solution was streaked onto XLD plate for *Salmonella* and TSAYE-NS for *E. coli* and incubated for 24 hr at 37°C. Additionally, 0.1 ml of enrichment solution was transferred to 10 ml universal pre-enrichment broth (Difco) for *Salmonella* and MacConkey broth (Difco) for *E. coli*, respectively, incubated at 37°C for 24 hr, and a loopful of the cultures was streaked onto XLD agar for *Salmonella* and MacConkey agar for *E. coli* (Rall, Rall, Aragon, & da Silva, 2005).

2.5 | Sensory evaluation after 12-week frozen storage

Sensory evaluation (overall appearance, color, texture, and aroma) was conducted using a 5-point subjective scale. For overall appearance, the following scale was used: 5, excellent quality, fresh appearing; 4, good quality, minor defects; 3, fair quality; 2, poor quality, excessive defects; 1, extremely poor quality, not usable. For color, texture, and aroma, similar 5-point scales were used. Ten untrained panelists performed subjective assessments for all samples. Frozen blueberry samples for each panelist were enclosed in a 20-ml plastic container, allowed to thaw for 15 min at ambient temperature before being presented to the panelists. Each panelist evaluated six blueberries from each treatment.

2.6 | Physical properties and total bacteria, yeasts, and molds counts of blueberries during frozen storage

Color, texture, and pH of uninoculated blueberries were determined at 0, 1, 2, 4, 8, and 12 weeks. Color was determined by a color reader (MINOLTA model CR-10, Minolta Camera Co., Ltd., Osaka, Japan) to determine the following color values: L^* (brightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness). Measurements were taken at three different parts of treated and untreated uninoculated blueberries. Texture was measured by a TA.XT2 texture analyzer (Texture Technology Corp., Scarsdale, NY) using a TA-91 Kramer shear probe with a rounded end. For the pH measurement, 5 g blueberries were placed in sterile stomacher sample bags and homogenized in a stomacher for 2 min at medium speed. The pH values of homogenates were measured by a pH meter (FiveEasy FE20, Mettler-Toledo AG, Greifensee, Switzerland).

The survival of total bacteria and yeasts and molds on uninoculated blueberries during 12-week frozen storage was also determined. Total bacteria account was enumerated on TSA plates and incubated at 37°C for 24 hr. Yeasts and molds were enumerated on Potato Dextrose Agar (Difco) acidified to a final pH of 3.5 with tartaric acid. The plates were incubated at 25°C for 3–5 days (Tournas, Stack, Mislivec, Koch, & Bandler, 2001).

2.7 | Total phenolic and anthocyanins content

The total phenolic and anthocyanins contents of untreated and treated blueberries were determined as described by Li and Wu (2013). For determining total phenolic content, the gallic acid was used as a standard. Twenty microliters of supernatants from centrifuged homogenates of treated and untreated blueberries, 180 μl of distilled water, 100 μl of the Folin–Ciocalteu reagent, and 0.5 ml of a 20% sodium carbonate (Sigma–Aldrich, Inc) solution composed the reaction mixtures. The reaction mixtures were covered, vortexed vigorously and allowed to react at room temperature for 2 hr in microcentrifuge tubes. Two hundred microliters of these mixtures was added into each blank well of a clear 96-well plate. The absorbance was measured at 765 nm by the Synergy 2 multimode microplate reader (BioTek Instruments, Inc., Winooski, VT) and the total phenolic content was calculated as mg gallic acid (Sigma–Aldrich) equivalent/100 g berries.

For determining total anthocyanins content, fresh blueberry samples at 5 g and 30 ml of 80:20 (vol/vol) methanol–water solution containing 0.1 ml/L acetic acid were added to a tube and homogenized by ultra-turrax for 2 min. The mixture (total volume of 30 ml) was placed in the dark for 1 hr and then sonicated for 15 min. After centrifugation at 5,000 rpm for 30 min at room temperature, the volume of the supernatants was recorded. The total anthocyanins content was measured by the pH differential method with some modification. The anthocyanin extract was dissolved in a 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) with a dilution factor at 6.0. The absorbance of each dilution was measured at 510 and 700 nm. The absorbance (A) of the diluted sample was calculated by the following formula:

$$A = (A_{510} - A_{700})\,\text{pH}\,1.0 - (A_{510} - A_{700})\,\text{pH}\,4.5$$

The monomeric anthocyanin pigment concentration in the original sample was expressed in equivalence of cyaniding-3-glucoside according to the following formula:

$$\text{Anthocyanin content}\,(\text{mg/L}) = (A \times \text{MW} \times \text{DF} \times 1000)/\varepsilon \times 1$$

where MW is the molecular weight of cyanidin-3-glucoside 449.2, DF is the dilution factor, and ε is the molar absorptivity, which equal to 26, 900 for cyanidin-3-glucoside.

2.8 | Statistical analysis

All experiments were conducted in three independent trials. The data are represented as mean values ± *SD*. Microbial survivors after

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treatments, color, pH measurements, and nutrient contents were analyzed for significant treatment differences by one-way analysis of variance, fit model test of JMP (v. 10.0, SAS Institute, Inc., Cary, NC). The different effect in washing experiments was assessed by fit model. Significance was determined at p values of .05 using Student's t test. Log reductions were calculated as difference between mean log *Salmonella* or *E. coli* population of unwashed samples and log survivor population of each treated sample.

3 | RESULTS AND DISCUSSION

3.1 | Combination of SDS and organic acids or hydrogen peroxide on inactivation of *Salmonella* and *E. coli* during frozen storage

Figure 1a shows the effect of washing and 12 weeks of frozen storage on survival of *Salmonella* on blueberries. The initial microbial load on

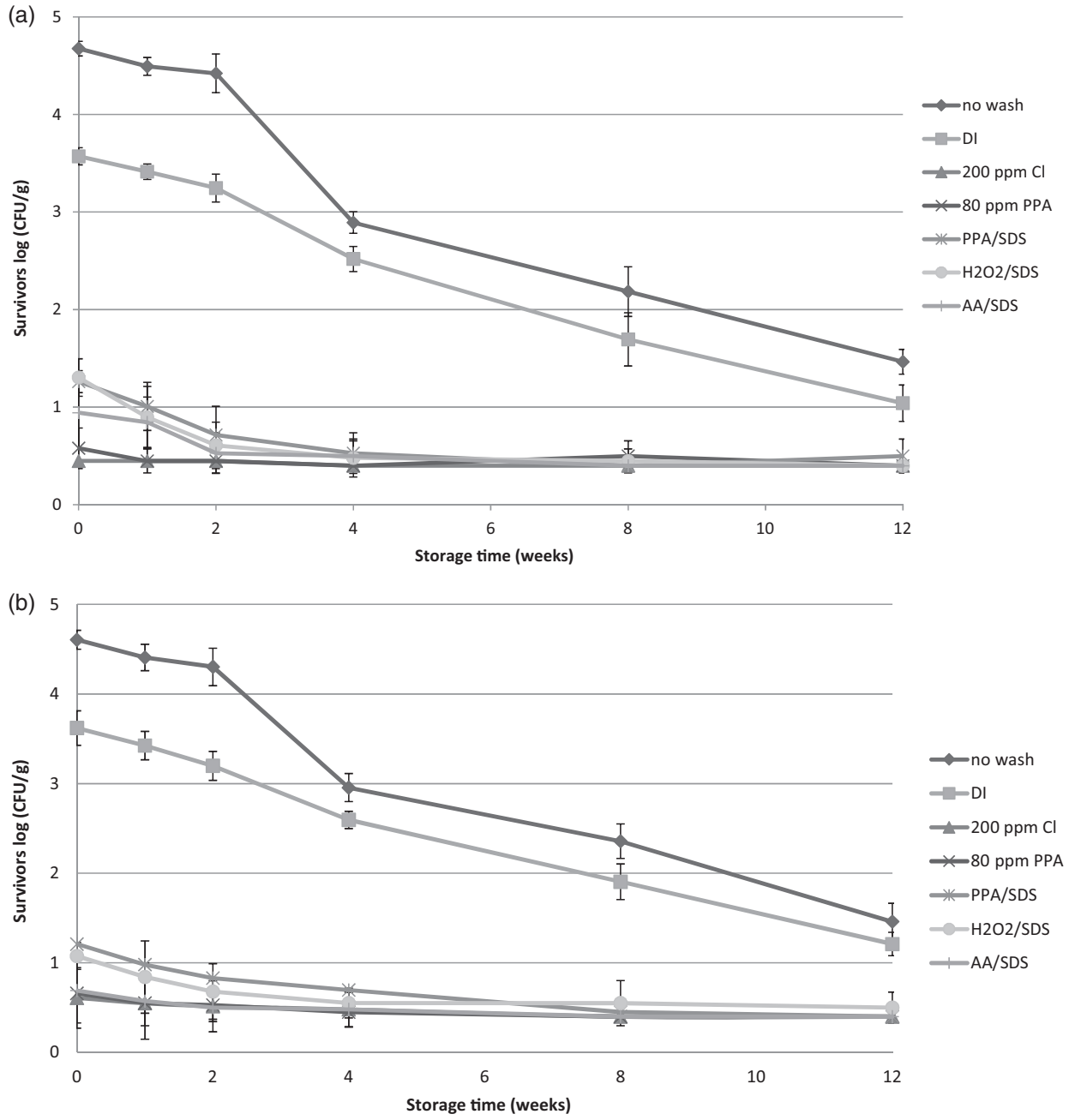

FIGURE 1 Pathogen survivors by washing blueberries contaminated with *Salmonella* (a) and *Escherichia coli* O157:H7 (b) with SDS and organic acids or hydrogen peroxide followed by 12 weeks of frozen storage. Microbial counts represent the mean and *SD*s of three independent trials. AA/SDS: 500 ppm acetic acid plus 5,000 ppm SDS; H_2O_2/SDS: 200 ppm hydrogen peroxide containing 5,000 ppm SDS; PPA/SDS: 20 ppm peroxyacetic acid combined with 5,000 ppm SDS. Detection limit was at 0.39 $\log_{10}$ CFU/g, indicated as a solid line in the figure. SDS, sodium dodecyl sulfate

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untreated and inoculated control samples was 4.7 $\log_{10}$ CFU/g. Washing the inoculated blueberries with the combinations of antimicrobials reduced *Salmonella* counts by 3.3–3.7 $\log_{10}$ CFU/g. However, no significant differences were detected among the three combinations ($p > .05$). The solution containing 80 ppm peroxyacetic acid reduced the population of *Salmonella* from 4.7 $\log_{10}$ CFU/g to 0.6 $\log_{10}$ CFU/g, a reduction similar to that with a 200-ppm chlorine wash ($p > .05$).Our results indicate that addition of 5,000 ppm SDS into 500 ppm acetic acid resulted in a 3.7-log reduction of *Salmonella*, which is in good agreement with previous research.

Further reduction in *Salmonella* number with three combination washing was noticed after frozen storage for 2 weeks as AA/SDS, PPA/SDS, and H_2O_2/SDS reduced viable counts of *Salmonella* by 4.1, 4.0, and 3.9 $\log_{10}$ CFU/g, respectively, which was a significant reduction compared to the control (0.5 $\log_{10}$ CFU/g reduction) ($p < .0001$). There was no significant difference in the effectiveness of the three combination washing solutions and treatment with 200 ppm chlorine after 2-week frozen storage ($p > .05$). After 8 weeks of frozen storage, the *Salmonella* counts with three combination washing treatments decreased to about 0.4 $\log_{10}$ CFU/g. After 12 weeks of frozen storage, no survivors among three combination washing treatments were detected by direct plating. However, *Salmonella* was detected in the enrichment cultures. *Salmonella* spp. are known for their tolerance to freezing (Archer, 2004). *Salmonella* Typhimurium survived on frozen fish and stored at $-17.9^{\circ}C$ for over 1 year with only 1 $\log_{10}$ reduction in numbers (Raj & Liston, 1961). *Salmonella* Typhimurium cells on frozen sausage and minced beef were only sublethally damaged after frozen storage at $-18^{\circ}C$ for up to 10 weeks (Barrell, 1988). Therefore, our control sample results with 0.5 $\log_{10}$ CFU/g reduction are consistent with previous studies that *Salmonella* cannot be eliminated during frozen storage. Our new washing solutions can produce further reduction in *Salmonella* at approximately 4 $\log_{10}$ CFU/g.

When organic acids or hydrogen peroxide plus SDS combination treatments were evaluated for killing *E. coli* O157:H7 on blueberries during frozen storage (Figure 1b), washes with AA/SDS for 60s reduced *E. coli* cell numbers by 3.9 $\log_{10}$ CFU/g, whereas PPA/SDS treatment reduced *E. coli* by 3.4 $\log_{10}$ CFU/g in comparison to the no-wash control. Similarly, *E. coli* was reduced by 3.5 $\log_{10}$ CFU/g when treated with H_2O_2/SDS for 60 s. Treatment with AA/SDS showed similar reductions to those obtained with 200 ppm chlorine ($p > .05$). After 1-week frozen storage, no significant differences were detected not only among three combination wash solutions tested ($p > .05$) but also between each combination wash solution and 200 ppm chlorine ($p > .05$). Further slightly reductions in *E. coli* numbers were observed during frozen storage for up to 12 weeks. For example, AA/SDS reduced *E. coli* numbers from 4.6 to 0.6 $\log_{10}$ CFU/g with 1-week frozen storage, whereas AA/SDS was able to reduce *E. coli* numbers from 0.6 to 0.4 $\log_{10}$ CFU/g during frozen storage from 1 week to 8 weeks. After 12-week frozen storage, *E. coli* on blueberries treated with the combination wash solutions was detectable by enrichment culture but not by directly plating. Our no-wash control results are in good agreement with Doyle and Schoeni's finding (Doyle & Schoeni, 1984) that *E. coli* O157:H7 survive well on food products during frozen storage.

3.2 | Sensory analysis

Freezing significantly affected the overall appearance of blueberries when compared with fresh ones ($p < .05$) (Table 1). No significant difference in the overall appearances were observed among the berries that underwent combination treatments, 200 ppm chlorine or DI washes prior to frozen storage ($p > .05$). Freezing did not have a noticeable effect on color and aroma, but caused significant damage to the texture of both treated and nontreated blueberries.

3.3 | Physical properties and microbiological quality (total bacteria account and yeasts and molds counts) of blueberries during frozen storage

The effect of washing treatment on the physical properties (color and texture) during cold storage is shown in Figure 2. Color parameters

TABLE 1 Sensory test results for fresh blueberries and blueberries washed with antimicrobial solutions and frozen stored for 12 weeks

Washing treatment	Overall appearance score	Color score	Texture score	Aroma score
AA/SDS (frozen)	2.3 ± 0.67^{B}	4.3 ± 1.06^{A}	2 ± 1.05^{B}	4.3 ± 0.82^{A}
H_2O_2/SDS (frozen)	2.4 ± 0.84^{B}	4.1 ± 0.99^{A}	1.5 ± 0.53^{B}	3.5 ± 1.08^{A}
PPA/SDS (frozen)	2.3 ± 0.82^{B}	4.2 ± 1.14^{A}	1.4 ± 0.52^{B}	3.7 ± 0.95^{A}
80 ppm peroxyacetic acid (frozen)	1.9 ± 0.57^{B}	4.3 ± 0.82^{A}	1.6 ± 0.84^{B}	3.6 ± 1.07^{A}
200 ppm cl (frozen)	1.9 ± 0.99^{B}	3.8 ± 1.4^{A}	1.6 ± 0.84^{B}	3.1 ± 1.29^{A}
DI (frozen)	2.3 ± 1.06^{B}	3.8 ± 1.03^{A}	1.7 ± 0.95^{B}	3.6 ± 0.84^{A}
No wash (frozen)	2.7 ± 0.95^{B}	3.9 ± 0.57^{A}	2.9 ± 0.88^{B}	4.1 ± 0.74^{A}
Fresh blueberries	4.9 ± 0.32^{A}	3.4 ± 1.35^{A}	4.6 ± 0.52^{A}	3.3 ± 1.16^{A}

Note: Values are means ± *SD*s (scale of 5 to 1) ($n \sim 10$). Data followed by the same superscripts indicate was insignificantly different to that of no wash berries in the same column ($p > .05$). DI: distilled water washing; AA/SDS: 500 ppm acetic acid plus 5,000 ppm SDS; H_2O_2/SDS: 200 ppm hydrogen peroxide containing 5,000 ppm SDS; PPA/SDS: 20 ppm peroxyacetic acid combined with 5,000 ppm SDS.
Abbreviation: SDS, sodium dodecyl sulfate.

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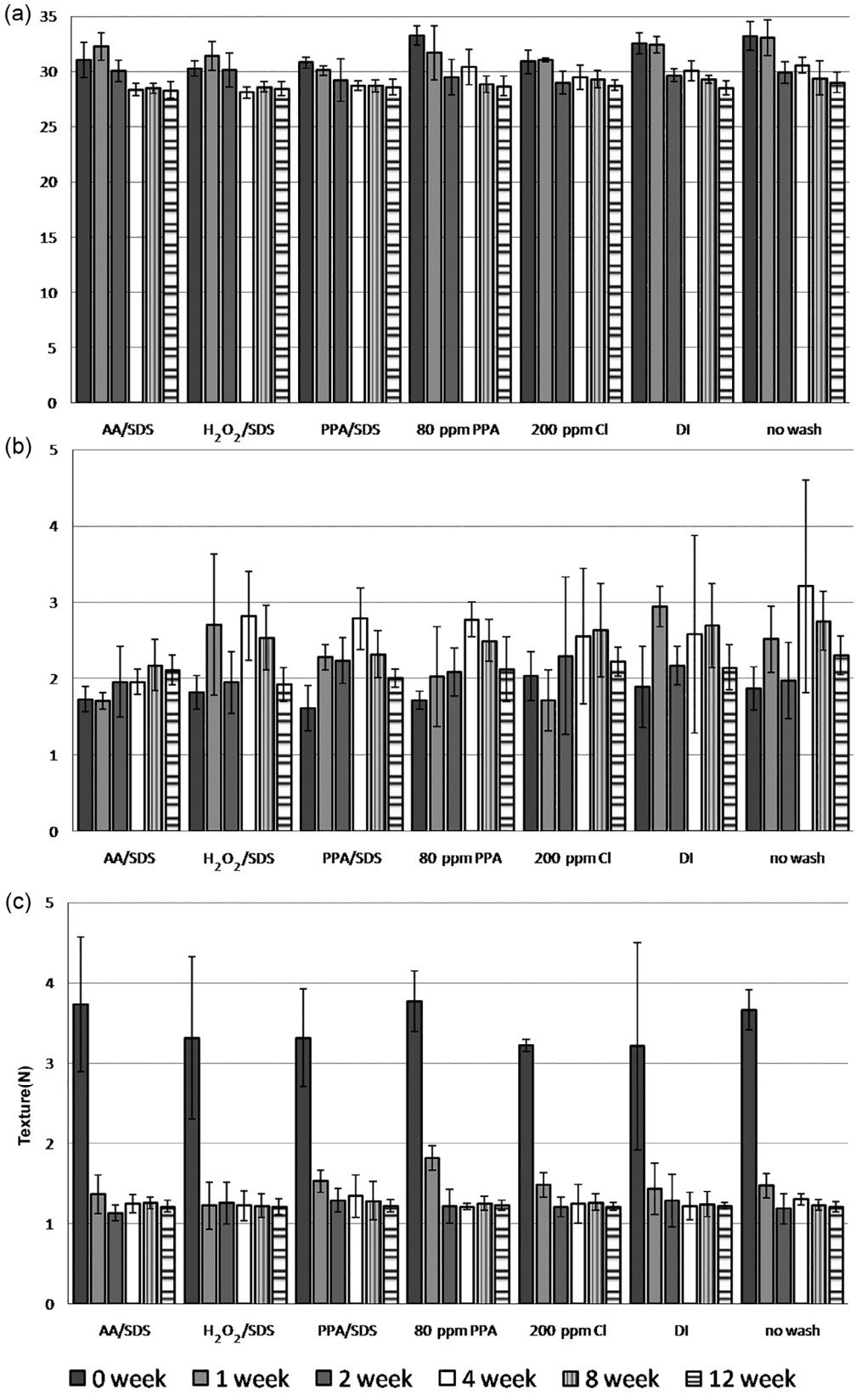

FIGURE 2 Changes of color (*L*) (a), color (chroma) (b), and texture (c) of blueberries during frozen storage. Data were expressed in the average ± the *SD* of three independent trials. AA/SDS: 500 ppm acetic acid plus 5,000 ppm SDS; H_2O_2 /SDS: 200 ppm hydrogen peroxide containing 5,000 ppm SDS; PPA/SDS: 20 ppm peroxyacetic acid combined with 5,000 ppm SDS. SDS, sodium dodecyl sulfate

(*L**, *a**, and *b**) were not significantly affected by washing treatments, whereas the value of *L** slightly decreased during frozen storage. The texture value of untreated blueberries was 3.6 N, while the texture values of treated blueberries were around 3.2 N at time 0. However, 1-week frozen storage decreased texture value of treated and non-treated blueberries to 1.2 N. Apparently, freezing caused severe

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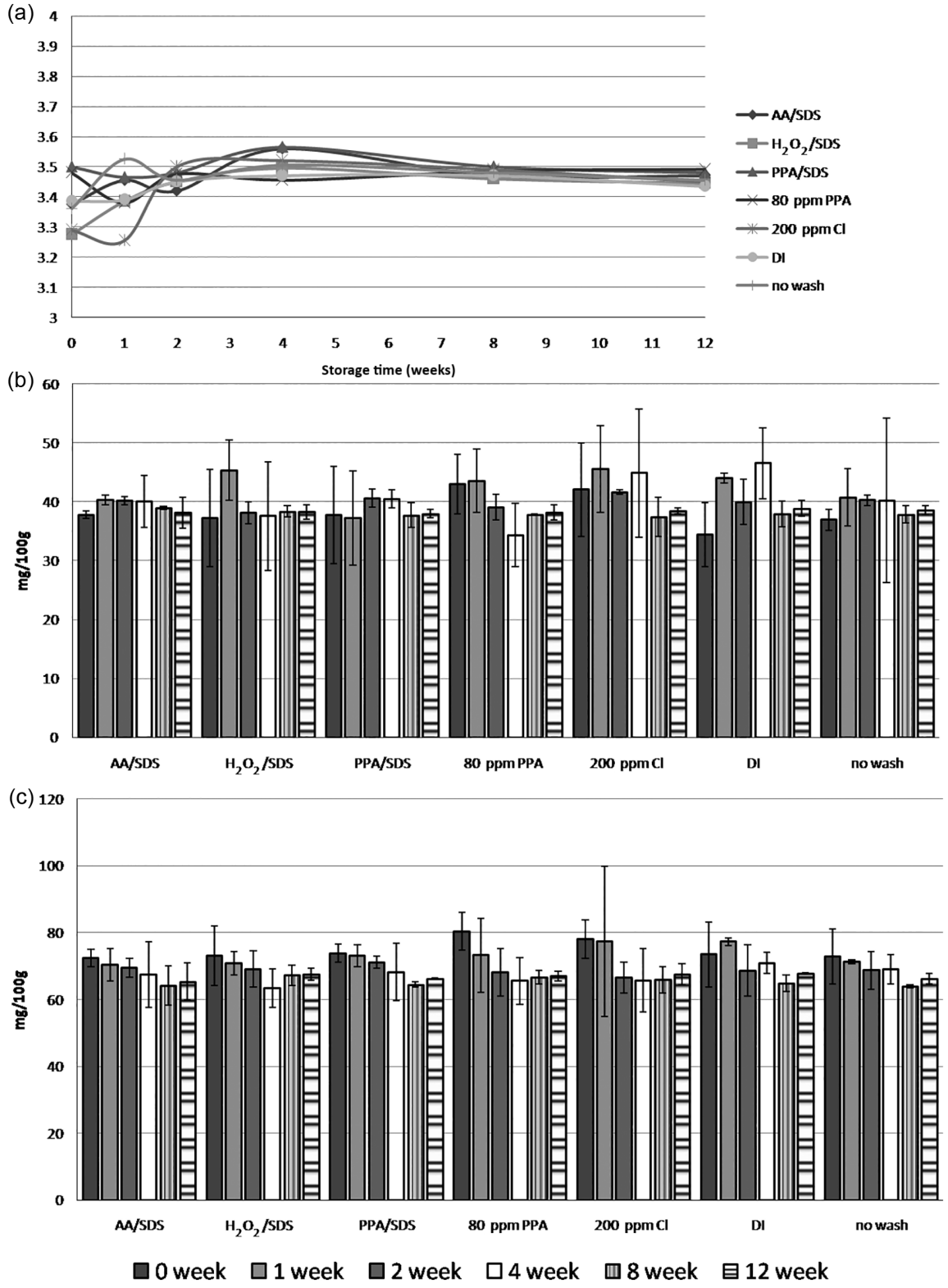

FIGURE 3 Changes of pH (a), total anthocyanins content (b), and total phenolic content (c) on blueberries during frozen storage. Data were expressed in the average ± the *SD* of three independent trials. AA/SDS: 500 ppm acetic acid plus 5,000 ppm SDS; H_2O_2/SDS: 200 ppm hydrogen peroxide containing 5,000 ppm SDS; PPA/SDS: 20 ppm peroxyacetic acid combined with 5,000 ppm SDS. SDS, sodium dodecyl sulfate

damage on the texture of treated and nontreated blueberries, which was related to the skin (epidermal and subepidermal) damage, flesh (parenchymal) damage, and leakage from the vascular tissue during frozen storage (Allan-Wojtas, Goff, Stark, & Carbyn, 1999). There was no significant difference in pH value between untreated and treated blueberries after washing or during the frozen storage ($p > .05$). For

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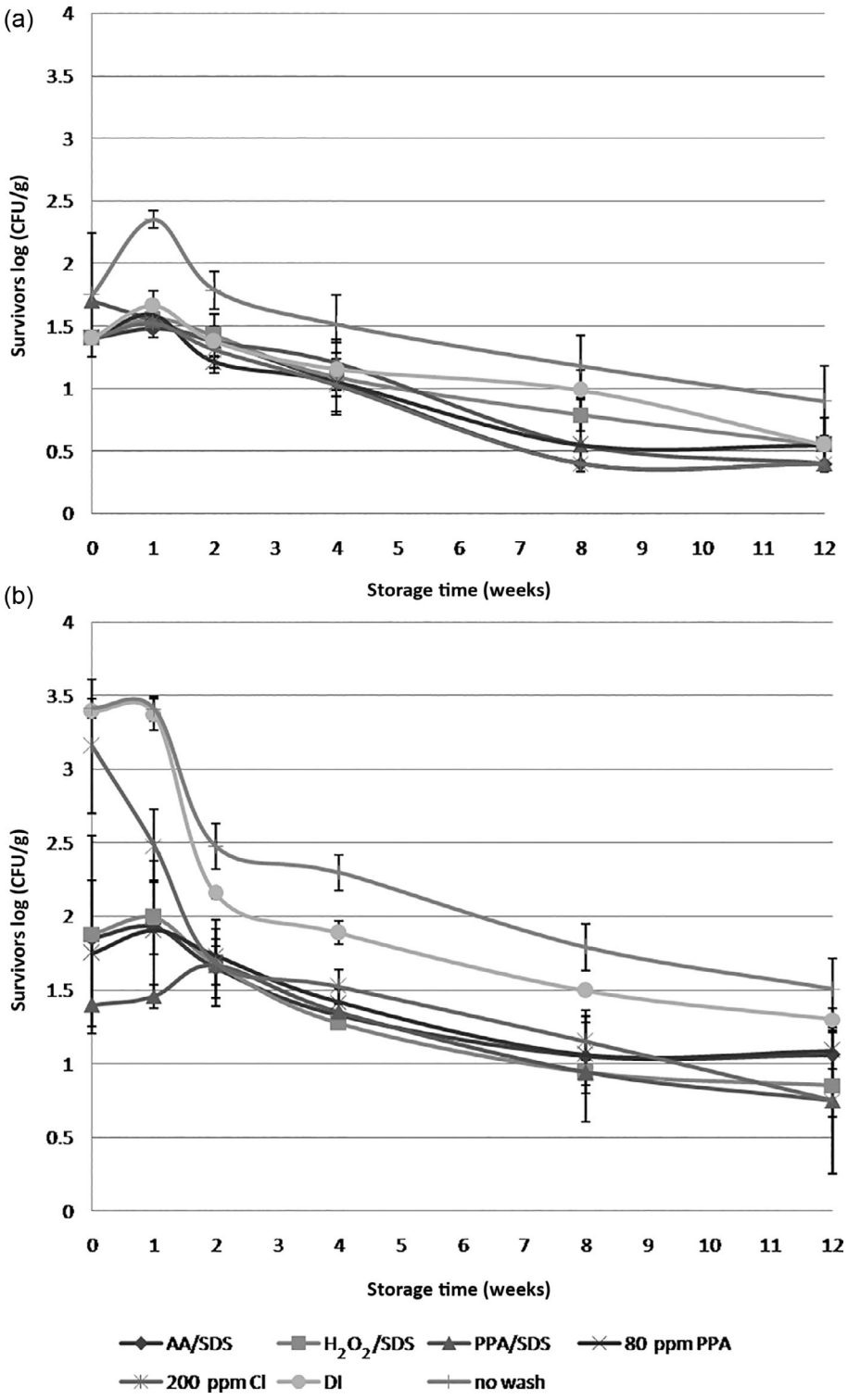

FIGURE 4 Inactivation of total bacteria (a) and molds and yeasts (b) during frozen storage after treatments with a combination of SDS and organic acids or hydrogen peroxide. Microbial counts represent the mean and *SD*s of three independent trials. AA/SDS: 500 ppm acetic acid plus 5,000 ppm SDS; H_2O_2/SDS: 200 ppm hydrogen peroxide containing 5,000 ppm SDS; PPA/SDS: 20 ppm peroxyacetic acid combined with 5,000 ppm SDS. SDS, sodium dodecyl sulfate

example, the pH value of untreated blueberries before frozen storage was 3.36, whereas the pH value of treated blueberries before frozen storage was around 3.38. With 12-week frozen storage, the pH value of untreated blueberries was 3.45, while the pH value of treated blueberries was around 3.46. No significant difference was detected in total anthocyanins and total phenolic content between untreated and treated blueberries at time 0 ($p > .05$) as the total anthocyanins and phenolic contents of untreated blueberries were 36.9 and 72.9 mg/100 g while the total anthocyanins contents of treated blueberries were around 37.7 mg/100 g, and the total phenolic content of treated blueberries ranged from 72.3 to 80.4 mg/100 g. After 12-week frozen storage, only small changes in the total anthocyanins and phenolic contents were observed (Figure 3). The total anthocyanins and total phenolic content of untreated blueberries was respectively increased to 38.2 mg/100 g and decreased to 66.2 mg/100 g, whereas the total anthocyanins and the total phenolic contents of

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treated blueberries were around 38.1 and 67.2 mg/100 g, respectively. Furthermore, no significant difference was observed in total anthocyanins and phenolic contents between each combination treatment and 200 ppm chlorine or DI wash. These findings agreed well with previous findings on nonsignificant decrease in anthocyanin levels of the frozen blueberry samples during 3 months of storage at −20°C (Lohachoompol, Srzednicki, & Craske, 2004). Therefore, in our present study, the use of three washing solutions, AA/SDS, PPA/SDS, and H_2O_2/SDS, did not have any negative effect on the physical and chemical properties of blueberries during the 12-week frozen storage.

The initial total bacteria and yeast and mold counts on unwashed blueberries were about 1.8 and 3.4 $\log_{10}$ CFU/g, respectively (Figure 4). The combination treatments (AA/SDS, PPA/SDS, and H_2O_2/SDS) resulted in total bacteria and yeasts and molds counts that were significantly lower than those of unwashed blueberries. The mean reductions by combination treatments from total bacteria and yeasts and molds counts were 0.4 and 1.5 $\log_{10}$ CFU/g, respectively, but these values were not statistically different at time 0. However, 200 ppm chlorine showed reduced effectiveness at inactivation molds and yeasts at time 0. Crowe et al. (2005) reported a 5 min spray of 100 ppm chlorine reduced populations of bacteria, yeast, and mold on blueberries by only 0.83, 0.77, and 0.61 $\log_{10}$ CFU/g, respectively. The reduced effectiveness of chlorine at inactivating surface microorganisms in previous research may be a result of organic matter surrounding the target cells. Further reduction in total bacteria and yeast and mold counts with the combination washes was noticed after frozen storage for 2 weeks. Additionally, all combination treatments had significantly lower total bacteria and yeast and mold counts than those of unwashed or DI-washed blueberries ($p < .05$). After 12-week frozen storage, the counts for total bacteria and molds and yeasts with the combination washing treatments decreased to about 0.5 and 0.8 $\log_{10}$ CFU/g, respectively. Moreover, there was no significant difference in the effectiveness of these three combination washing solutions and 200 ppm chlorine on the inactivation of total bacteria and molds and yeasts during frozen storage ($p > .05$). Therefore, AA/SDS, PPA/SDS, and H_2O_2/SDS showed effective removal and inactivation of total bacteria and molds and yeasts on blueberries.

4 | CONCLUSION

In conclusion, washing blueberries with 500 ppm acetic acid plus 5,000 ppm SDS (AA/SDS), 20 ppm peroxyacetic acid combined with 5,000 ppm SDS (PPA/SDS), or 200 ppm hydrogen peroxide in combination with 5,000 ppm SDS (H_2O_2/SDS) lowered the surviving populations of attached *Salmonella* Typhimurium and *E. coli* O157:H7 after 12 weeks of frozen storage. Additionally, the superior effect of these three washing solutions on removing and inactivating total bacteria and molds and yeasts was noticeable. However, *Salmonella* Typhimurium and *E. coli* O157:H7 cannot be eliminated and survive well during frozen storage. Although these washings did not have any negative effect on the physical and chemical properties of blueberries at time 0, frozen storage caused significant damage on the texture of both treated and nontreated blueberries. Results from this study, therefore, may provide the blueberry industry with important information to assist in selection of effective antimicrobial strategies and improve the safety of frozen blueberries.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

We will share our original data upon request as appropriate and necessary.

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