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Potential of acidified sodium benzoate as an alternative wash solution of cherry tomatoes: Changes of quality, background microbes, and inoculated pathogens during storage at 4 and 21°C post-washing

Huaiqiong Chen, Yue Zhang[1], Qixin Zhong*

Department of Food Science, The University of Tennessee, Knoxville, TN, USA

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ABSTRACT

To evaluate the feasibility of acidified sodium benzoate (NaB) as alternative washing solutions of fresh produce, the survival of inoculated pathogens, the background molds and yeasts counts, and quality parameters were compared during 4 and 21 °C storage of cherry tomatoes washed with 3000 ppm NaB at pH 2.0, 200 ppm free chlorine at pH 6.5, water adjusted to pH 2.0, and distilled water. The acidified NaB solution was the most effective in reducing the population of *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* cocktails on tomatoes (> 4 log CFU/g). NaB was more effective than free chlorine ($P < 0.05$) in reducing the two Gram-negative bacteria on tomatoes, while the reduction of Gram-positive *L. monocytogenes* by NaB (5.49 log CFU/g) and chlorine (4.98 log CFU/g) was similar ($P > 0.05$). No recovery of bacteria was found in all treatments during storage for 15 days. The acidified NaB effectively controlled yeasts and molds on cherry tomatoes to < 1 log CFU/g or below the detection limit at both temperatures during 15-d storage, while free chlorine did not. Compared to unwashed controls, NaB had no effect on color, weight loss, firmness, and total soluble solids content of tomatoes during storage. The effect of NaB reducing pathogenic and spoilage microorganisms on tomatoes and maintaining quality during storage suggests its potential as an alternative wash solution in postharvest processing of fresh produce.

1. Introduction

The consumption of fresh produce has been increasing due to consumer demand of healthy diets. As a result, the microbial safety of fresh produce becomes an increasing concern because of the potential increase in outbreaks of foodborne illnesses due to pathogens that contaminate fresh produce and survive and proliferate at complicated conditions from farm to fork (Gil et al., 2009; Joshi et al., 2013). This concern is supported by statistical data. In 1998–2007, fresh produce was associated with 684 outbreaks involving 26,735 cases of illnesses (DeWaal et al., 2009). The foodborne illnesses resulting from contaminated produce were estimated to cost nearly $ 39 billion annually in the US and account for approximately 26% of the total economic loss caused by foodborne illnesses (Scharff, 2010). Therefore, more work is needed to improve the microbial safety of produce.

Tomatoes are one of the most commonly consumed vegetable products and contain several health-promoting compounds, including vitamins A, C, E and several carotenoids (Beecher, 1998; Tommonaro et al., 2008). The frequent consumption of tomatoes is a factor making this produce commodity accounting for about 17.1% of all produce-related outbreaks in 1996–2008 (Gravani, 2009). Washing with tap water containing a sanitizer such as chlorine is often used to visually clean produce but is limited in the effectiveness decontaminating pathogens (Joshi et al., 2013). For example, chlorinated water results in only about 1–2 log reduction of most bacteria on fresh produce (Mukhopadhyay et al., 2014).

The search to replace chlorine for the improved efficacy to decontaminate fresh produce has been studied for many compounds, including organic acids that are generally-recognized-as safe (GRAS). Antimicrobial activity of lactic, citric, acetic, and ascorbic acids dissolved at 0.5% and 1% in water was studied to reduce *Escherichia coli* (ATCC 25922) and *Listeria monocytogenes* (ATCC 7644) inoculated on iceberg lettuce after contacting for 2–5 min, and the respective maximum reduction of 2.0 and 1.5 log CFU/g was observed for *E. coli* and *L. monocytogenes* by 1.0% lactic acid (Akbas and Olmez, 2007). In another study, the efficacy of different organic acids in disinfecting whole red

* Corresponding author. Department of Food Science, The University of Tennessee, 2510 River Drive, Knoxville, TN, 37996, USA.
E-mail address: qzhong@utk.edu (Q. Zhong).
[1] Current address. Department of Food Science and Technology, University of Nebraska Lincoln, Lincoln, Nebraska, 68588, USA.

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apples and lettuce was compared (Park et al., 2011). The efficacy varied for the two produce products, and the treatments with 1–2% organic acids for up to 10 min resulted in a reduction of ~3 log CFU/apple or ~2 log CFU/g lettuce. Lactic acid at ~50 °C was observed to be more effective than at 40 and 21 °C in reducing bacteria on several produce (Dikici et al., 2015; Huang and Chen, 2011), with the highest reduction (4.0 log CFU/g) observed for spinach and soybean sprouts by 2.5% lactic acid at 50 °C (Dikici et al., 2015). Peroxyacetic acid has drawn much attention in recent years due to its strong antimicrobial activity, no by-product formation during treatment, and a low usage level, usually 20–80 ppm (Kitis, 2004). Its sanitation effect results from its dissociation products, hydrogen peroxide and acetic acids, both of which are effective antimicrobials (Kitis, 2004; Vandekinderen et al., 2009). In a study, 50 ppm peroxyacetic acid reduced *E. coli* O157:H7 inoculated on tomatoes by more than 4.5 log/g (Keeratipibul et al., 2011). In another study, 80 ppm peroxyacetic acid only reduced 2 log CFU/g of *E.coli* O157:H7 inoculated on apples (Wright et al., 2000). Most of these studies only reported log reductions after treatment of fresh produce. Few studies evaluated the survival of pathogens and quality changes of produce during storage, post washing.

To maintain produce quality during storage, antimicrobials effective against spoilage microorganisms can be applied. Ideally, these antimicrobials can also effectively decontaminate pathogens during washing produce. Sodium benzoate (NaB) is a low-cost GRAS additive used in foods at a concentration up to 0.1% and is known for its activities against a wide range of microorganisms, especially fungi, yeasts, and molds (Chipley, 2005). NaB is widely added in food products such as juices, soda, soy sauces, ketchups to prevent spoilage (Chipley, 2005; Gören et al., 2015). NaB in combination with citrus extract inactivated *Fusarium oxysporum* spores in pineapple juice (Bevilacqua et al., 2012). Recently, Montesinos-Herrero et al. (2016) reported the effectiveness of NaB controlling green and blue molds on citrus fruits but did not study the effectiveness against pathogens. The inhibition of *L. monocytogenes* in tryptic soy broth (TSB) by 0.05 or 0.1% (w/v) NaB at pH 5.0 was reported for at least 40 h, while the bacterium grew in the presence of 0.2% (w/v) NaB at pH 5.6 after 8 h incubation (Elshenawy and Marth, 1988). The anti-listerial activity of NaB was limited when tested at pH 5.6 (Elshenawy and Marth, 1988) and 7.0 (Buazzi and Marth, 1992). The pH-dependent activity of NaB confirms that benzoic acid, which is present at a higher concentration at a lower pH, is the active form of the antimicrobial against bacteria. This well-accepted theory was verified in our earlier study showing a greater activity of NaB against *E. coli* O157:H7, *Salmonella enterica* and *L. monocytogenes* in growth medium adjusted to a lower pH and the poor activity observed at pH 4.0–7.0 (Chen and Zhong, 2018). When rinsing cherry tomatoes inoculated with *E. coli* O157:H7 and *S. enterica* cocktails at 21 °C for 3 min, the wash solution with 3000 ppm NaB adjusted to pH 2.0 was significantly more effective than 200 ppm free chlorine at pH 6.5, while the two washes were similar in reducing *L. monocytogenes* cocktail on tomatoes. The acidified NaB wash solution was also observed to prevent cross-contamination of pathogens, and the organic load did not affect the washing treatments. However, the recovery of pathogens during storage following washing is to be studied, as well as impacts on tomato quality and native spoilage microorganisms. The hypothesis of the present work is therefore that highly acidified NaB solutions can be used to wash fresh produce to reduce pathogenic bacteria and spoilage yeasts and molds during washing and subsequent storage.

The first objective of the present study was to determine the survival of *E. coli* O157:H7, *Salmonella enterica* and *L. monocytogenes* inoculated on cherry tomatoes after washing with NaB acidified to pH 2.0 at room conditions and during storage at 4 and 21 °C. The second objective was to evaluate quality changes of washed tomatoes during storage. The third objective was to characterize native molds and yeasts after washing and during storage. The treatments were compared with 200 ppm free chlorine at pH 6.5, the highest chlorine concentration commonly used in the produce industry (Huang and Chen, 2011; Stopforth et al., 2008), water acidified to pH 2.0, and neutral water.

2. Materials and methods

2.1. Materials

NaB (> 98% purity) was obtained from Sigma-Aldrich Corp. (St. Louis, MO). HCl (12 N), peptone, yeast extract (Remel®™), TSB (Remel®™), Dichloran rose bengal chlortetracycline (DRBC, BD Difco) agar, polysorbate 80 (Tween 80), calcium hypochlorite, phosphate-buffered saline (PBS) at pH 7.2 (Becton, Dickinson and Company, Sparks, MD), and blender bags (105 mm × 155 mm) were purchased from Thermo Fisher Scientific Inc. (Pittsburgh, PA). Agar was a product of Sigma-Aldrich Corp. (St. Louis, MO, USA). Nalidixic acid (NA, Fisher Scientific Inc., Pittsburgh, PA) was prepared in 100% ethanol at 1600 ppm as a stock solution. NatureSweet® cherry tomatoes (San Antonio, TX) were purchased from a local supermarket.

2.2. Preparation of bacterial inocula

Cocktails of bacterial strains/serovars were prepared to inoculate tomatoes in this study. Each bacterial cocktail was composed of five strains/serotypes isolated from outbreaks: H1730, F4546, K3995, CDC658, and 932 for *E. coli* O157:H7; ENV2011010804-1 (390–1), ENV2011010804-2 (390–2), 310, Scott A, and V7 for *L. monocytogenes*, and Agona, Montevideo, Gaminara, Michigan, and Saint Paul for *S. enterica*. The stock cultures were obtained from the collection of the Department of Food Science at the University of Tennessee (Knoxville, TN, USA). All bacterial strains were individually made resistant to 40 ppm NA and maintained at −20 °C in 40% glycerol. All TSB and TSA media were supplemented with 40 ppm NA as TSBN and TSAN, respectively. The media for *L. monocytogenes* were additionally added with 0.6% yeast extract.

Each strain was sub-cultured twice in TSBN with an interval of 24 h. Subsequently, a 300 μL aliquot of each culture was spread onto TSAN and incubated at 37 °C for *E. coli* O157:H7 and *S. enterica* and 32 °C for *L. monocytogenes* for 24 h. The bacterial lawn was rehydrated with 5 mL (3 mL for first time + 2 mL for second time) PBS with 0.2% Tween 80. The liquid was collected from the TSAN plates using a spreader. The cocktail was then prepared using 5 mL of the collected liquid of each strain to obtain a total volume of around 25 mL as the culture for inoculating tomatoes. Microbial counts in these cultures were ~10^{11}, 10^{12}, and 10^{13} CFU/mL for *L. monocytogenes*, *E. coli* O157:H7, and *S. enterica*, respectively.

2.3. Sample inoculation and washing

Washing solutions were all prepared with distilled water and included 3000 ppm NaB adjusted to pH 2.0 with 6.0 N HCl, 200 ppm free residue chlorine at pH 6.5, water adjusted to pH 2.0 with 6.0 N HCl, and water at neutral pH. The fresh chlorine solution was prepared right before washing by dissolving calcium hypochlorite (~65% available chlorine) in distilled water and adjusting pH to 6.5 with 1.0 N HCl.

On the day of experiments, cherry tomatoes were purchased from a supermarket and were used directly. Each tomato was spot inoculated with 100 μL (10 μL/spot) of a bacterial cocktail. Tomatoes were dried inside a biosafety cabinet for 2–3 h. Ten inoculated tomatoes were washed with 400 mL of each washing solution for 3 min at room temperature (RT, ~21 °C). For chlorine treatments, the free residual chlorine was monitored using a Free Chlorine & Chlorine Ultra High Range ISM meter (Hanna Instruments, Woonsocket, RI) before use.

2.4. Microbial survival during storage

The ten inoculated tomatoes, after washing and drying in a biosafety cabinet, were packed in a vented plastic fruit container (Katgely

Inc., Brooklyn, NY). Control tomatoes without any washing treatment were packed directly. To evaluate the growth of yeasts and molds after washing and during storage, un-inoculated tomatoes were washed and then packed as above. The containers with cherry tomatoes were stored at 4 °C in a refrigerator and at RT on the bench for 0, 2, 5, 10 and 15 d. One container was treated as an independent replicate, and a total of 160 containers for 2 temperatures × 5 wash treatments × 4 microbial treatments (3 bacteria and 1 for total molds and yeasts) × 4 replications were used. Two tomatoes (considered as one sample) from each container were randomly sampled at a time point for bacteria enumeration. The 2 sampled tomatoes (~20 g) were placed in a blender bag and 50 mL PBS with 0.2% Tween 80 was added. After hand-massaging for 15 s, the liquid was serially diluted in 0.1% peptone before spread plating in duplicate on TSAN plates, which resulted in a total of 8 measurements (4 replicates × duplicate sampling) for microbial population at each time point. The massaging time of 15 s was decided according to a preliminary comparison on massaging duration to remove *E.coli* O157:H7 cocktail from inoculated tomato surface. The recovered *E. coli* O157:H7 population was 7.92 ± 0.19, 8.00 ± 0.19, and 8.15 ± 0.09 log CFU/g, respectively, for treatments massaged for 15, 30, and 45 s, which were not different among each other ($p > 0.05$). The viable *L. monocytogenes*, *E.coli* O157:H7, and *S. enterica* were determined by enumeration on TSAN plates after incubation at the respective optimum temperature for 24 h as above. The liquid spread on the DRBC agar was incubated at RT for 5 days before enumeration of yeasts and molds.

2.5. Quality changes of tomatoes during storage

Tomatoes without bacterial inoculation were washed and packaged as above, with some modifications on the number of tomatoes per container. Six tomatoes were labeled with numbers, packed in a container, and used to determine both color and weight loss of each tomato. Separate tomatoes were used in determination of firmness and total soluble solids (TSS) content. For firmness determination, 25 tomatoes were packed in a container, and the same tomatoes after the firmness determination were used to measure TSS content. After storage at 4 °C and RT for 0, 2, 5, 10 and 15 d, the following parameters were determined.

2.5.1. Color

Surface color of tomatoes was evaluated using a HunterLab Miniscan® XE plus colorimeter (Hunter Associates Laboratory Inc., Reston, VA) to obtain CIE LAB values: $L^* =$ lightness; $a^* =$ green/red hue component; $b^* =$ yellow/blue hue component. In addition, in order to compare samples with different initial color values, total color differences (ΔE^*) were calculated using Eq. (1):

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

where L_0^*, a_0^*, and b_0^* are the color values of the control on day 0.

2.5.2. Weight loss

Weight loss of tomatoes was determined by measuring the mass of containers after a storage duration (W), compared to the mass at day 0 (W_0), as in Eq. (2).

$$\text{Weight loss (\%)} = \frac{W_0 - W}{W_0} \times 100 \quad (2)$$

2.5.3. Firmness

The firmness of cherry tomatoes was evaluated at RT using a texture analyzer (TA-XT Plus®, Texture Technologies Corp., Scarsdale, NY) equipped with a cone-shaped puncture probe (diameter = 2 mm). The penetration test was performed at a speed of 3 mm/s and a penetration distance of 10 mm (Pinheiro et al., 2013). Force-distance curves were recorded and the maximum peak force (N) was used as an indicator of firmness. At each storage time point, five fruits were measured for each treatment.

2.5.4. Total soluble solids (TSS) content

The tomatoes used to measure firmness were then used to determine the TSS content. A juice drop was prepared from a tomato by hand squeezing the fruit and the TSS content was determined with a hand-held refractometer (model TS400 m, Reichert Inc., Buffalo, NY) (Wills and Ku, 2002).

2.6. Data analysis

All results were reported as mean ± standard deviation. According to the descriptions above, a total of 8 replicates for microbial experiments, 6 replicates for color measurements, and 5 replicates for the firmness and TSS measurements were conducted. Statistical analyses were conducted with SAS 9.4 software (SAS Institute Inc.; Cary, NC) using the Mixed Model Analysis of Variance with the GLIMMEX procedure ($P < 0.05$) and Tukey's test. One-way ANOVA with Tukey's test was used to compare the recovered microbial populations after the three massaging durations ($n = 3$).

3. Results and discussion

3.1. Survival of pathogens on cherry tomatoes after washing and during storage

High levels of initial inoculation of pathogens were applied in this study to better distinguish treatments after washing and during storage. The survival bacterial populations on cherry tomatoes treated with various washing solutions for a contact time of 3 min and stored at 4 and 21 °C for up to 15 d are presented in Tables 1 and 2, respectively. The bacterial population of the unwashed control groups decreased significantly over time ($P < 0.05$), particularly at day 2. This can be attributed to the sudden change of nutritional and environmental conditions around the bacteria (Knudsen et al., 2001), because the surface of fruits is dry and the storage temperature is not the optimum for bacterial growth. Similar observations were reported for bacteria on strawberries (Knudsen et al., 2001) and cantaloupes (Zhang et al., 2015). Overall, the two Gram-negative bacteria survived better than *L. monocytogenes* during storage, which agrees with the reports that *Salmonella* (Asplund and Nurmi, 1991) and *E. coli* O157:H7 (Eribo and Ashenafi, 2003) can grow well on tomatoes, although tomatoes are not a good reservoir of *L. monocytogenes* (Beuchat and Brackett, 1991). These characteristics may explain why most outbreaks linked to tomatoes are associated with *Salmonella* and *E. coli* O157:H7 (Valadez et al., 2012).

The neutral water treatment reduced the population of bacteria on tomatoes by 0.5–2 log CFU/g after 3-min washing, which agrees with studies of Stopforth et al. (2008) and Keskinen et al. (2009). Reduction of bacterial populations by neutral water in this study was due to the rinsing or washing-off effect of bacteria from cherry tomatoes. Rinsing can also change the microenvironment of bacteria (Huang et al., 2018; Keskinen et al., 2009). Therefore, although the pathogen population maintained at a relatively high level compared to other treatments, rinsing resulted in a significantly ($P < 0.05$) lower survival of bacteria than the unwashed control during storage (Tables 1 and 2), corresponding to a greater reduction rate.

The acidified water (pH 2.0) treatment resulted in a reduction of 2–3 log CFU/g after washing and had a more significant effect ($P < 0.05$) than the neutral water treatment on bacterial survival during storage at both 4 and 21 °C (Tables 1 and 2), with an additional reduction of 2–3 log CFU/g observed for all bacteria during the extended storage. This may be due to residual protons further killing bacteria initially injured during washing.

Table 1
Changes in populations of *Listeria monocytogenes* (1a), *Escherichia coli* O157:H7 (1b) and *Salmonella enterica* (1c) cocktails on tomatoes washed with different solutions and stored at 4 °C for up to 15 d. The detection limit was 0.4 log CFU/g. [a]

Time (d)	Control	Neutral distilled water	Water, pH2.0	Chlorine, 200 ppm	NaB, 3000 ppm
Table 1a					
0	7.93 ± 0.16 aA	6.09 ± 0.07bA	5.25 ± 0.41bA	2.43 ± 1.20 cA	2.95 ± 0.95 cA
2	6.49 ± 0.14 aB	3.87 ± 0.40bB	2.61 ± 0.92bcB	1.51 ± 1.62cAB	1.76 ± 1.03cAB
5	6.11 ± 0.21 aB	3.10 ± 0.42bC	1.72 ± 0.82cBC	< 0.40 dB	1.79 ± 1.19cAB
10	5.48 ± 0.26 aC	2.74 ± 0.82bC	1.93 ± 1.10bBC	1.55 ± 1.67bAB	1.51 ± 1.09bAB
15	5.08 ± 0.45 aC	1.77 ± 0.46bD	1.01 ± 0.42bcC	< 0.40 cB	1.42 ± 0.91bB
Table 1b					
0	8.26 ± 0.09 aA	7.50 ± 0.13bA	6.57 ± 0.35 cA	5.56 ± 0.67 dA	1.66 ± 0.51eA
2	6.98 ± 0.17 aB	6.18 ± 0.29 aB	3.31 ± 1.27bB	3.65 ± 0.84bB	0.92 ± 0.75cAB
5	6.93 ± 0.31 aB	5.67 ± 0.21bC	1.25 ± 0.73 dC	2.23 ± 0.85 cC	< 0.4eB
10	6.26 ± 0.42 aC	5.14 ± 0.28bD	< 0.4 dC	1.44 ± 1.21 cC	< 0.4 dB
15	6.00 ± 0.34 aC	5.15 ± 0.46bD	< 0.4 dC	1.90 ± 1.06 cC	< 0.4 dB
Table 1c					
0	8.76 ± 0.07 aA	7.99 ± 0.30bA	5.56 ± 0.42 cA	7.62 ± 0.17bA	3.88 ± 0.76 dA
2	8.27 ± 0.06 aB	6.48 ± 0.41bB	3.69 ± 1.18 cB	6.08 ± 0.56bB	1.56 ± 0.36 dB
5	8.04 ± 0.11 aC	5.98 ± 0.10bC	2.62 ± 0.54 cB	5.96 ± 0.40bB	1.23 ± 0.81dBC
10	7.86 ± 0.10aD	5.93 ± 0.33bC	0.81 ± 0.49 dC	4.22 ± 0.73cD	0.99 ± 0.91dBC
15	7.69 ± 0.06 aE	5.47 ± 0.29bD	1.28 ± 0.96 cC	4.98 ± 0.42bC	< 0.4 dC

[a] Numbers are mean ± SD ($n = 8$). The lower-case letters after numbers are the significant difference over the row, while the capital letters are the significant difference over the column.

Table 2
Changes in populations of *Listeria monocytogenes* (a), *Escherichia coli* O157:H7 (b) and *Salmonella enterica* (c) cocktails on tomatoes washed with different solutions and stored at room temperature of ~21 °C for up to 15 d. The detection limit was 0.4 log CFU/g.[a]

Time	Control	Neutral distilled water	Water, pH2.0	Chlorine, 200 ppm	NaB, 3000 ppm
Table 2a					
0	7.76 ± 0.04 aA	6.89 ± 0.24bA	4.97 ± 0.58 cA	2.25 ± 0.87 dA	2.56 ± 0.18 dA
2	7.12 ± 0.37 aA	4.65 ± 0.32bB	2.49 ± 0.64 cB	1.79 ± 1.45cAB	1.56 ± 0.25cBC
5	5.65 ± 0.70 aB	3.35 ± 1.51bC	2.30 ± 0.72bcBC	1.49 ± 1.34cAB	1.97 ± 0.99bcAB
10	5.59 ± 0.58aBC	1.90 ± 0.76bD	1.46 ± 1.02bcC	< 0.4 dB	0.95 ± 0.41cdC
15	4.92 ± 0.37 aC	1.64 ± 0.46bD	1.46 ± 0.41bcC	0.93 ± 0.55cAB	1.25 ± 0.25bcBC
Table 2b					
0	7.77 ± 0.08 aA	7.28 ± 0.22 aA	5.19 ± 0.49bA	5.54 ± 0.66bA	1.81 ± 0.65 cA
2	7.29 ± 0.24 aA	5.84 ± 0.51bB	2.78 ± 0.63 cB	3.46 ± 1.09 cB	0.44 ± 0.11 dB
5	5.84 ± 0.33 aB	5.57 ± 0.32 aB	1.49 ± 0.84bcC	1.68 ± 0.94bC	0.78 ± 0.41 cB
10	5.21 ± 0.51 aC	4.85 ± 0.35 aC	1.31 ± 1.12bC	0.99 ± 0.56bcC	< 0.4 cB
15	4.59 ± 0.65aD	4.03 ± 0.72 aC	0.71 ± 0.39bC	0.63 ± 0.43bC	< 0.4bB
Table 2c					
0	8.60 ± 0.11 aA	7.96 ± 0.10 aA	5.81 ± 0.44bA	7.62 ± 0.17 aA	3.76 ± 1.27 cA
2	7.81 ± 0.20 aB	6.39 ± 0.16bB	3.20 ± 1.11 cB	5.87 ± 0.42bB	2.51 ± 0.91 cA
5	7.81 ± 0.13 aB	6.44 ± 0.37bB	2.78 ± 1.09 cB	5.50 ± 0.77bB	2.30 ± 0.89 cA
10	7.12 ± 0.33 aC	6.25 ± 0.35 aB	2.54 ± 0.67 cB	4.50 ± 0.52bC	2.55 ± 0.08 cA
15	6.44 ± 0.56aD	5.20 ± 0.47bC	2.20 ± 0.53 dB	3.88 ± 1.02 cC	2.27 ± 0.16 dA

[a] Numbers are mean ± SD ($n = 8$). The lower-case letters after numbers are the significant difference over the row, while the capital letters are the significant difference over the column.

Table 3
Total mold and yeast counts of tomatoes during storage for up to 15 d at 4 °C (a) and room temperature of ~21 °C (b) without (control) or with washing by different solutions. The detection limit was 0.4 log CFU/g.[a]

Time	Control	Neutral distilled water	Water, pH2.0	Chlorine, 200 ppm	NaB, 3000 ppm
Table 4a					
0	4.00 ± 0.11aBC	3.53 ± 0.71abA	2.81 ± 0.46bcA	2.41 ± 0.73 cA	0.66 ± 0.30 dA
2	3.95 ± 0.34 aC	2.80 ± 0.79bAB	1.62 ± 0.35 cB	1.97 ± 0.85bcA	1.12 ± 0.35 cA
5	4.22 ± 0.11 aB	2.28 ± 0.55bB	1.84 ± 0.44bB	0.83 ± 0.81 cB	0.65 ± 0.35 cA
10	4.26 ± 0.14 aB	2.90 ± 0.80bAB	1.98 ± 0.69 cB	1.80 ± 0.30 cA	0.57 ± 0.32 dA
15	4.58 ± 0.14 aA	2.26 ± 0.35bB	1.65 ± 0.88bB	1.66 ± 0.47bAB	0.74 ± 0.60 cA
Table 4b					
0	3.01 ± 0.26 aA	3.17 ± 0.34 aA	2.41 ± 0.82 aA	2.41 ± 0.73 aA	< 0.4bA
2	2.04 ± 0.57 aB	1.74 ± 0.32 aB	1.91 ± 0.50 aA	1.57 ± 0.95aAB	< 0.4bA
5	2.37 ± 0.61abAB	2.77 ± 0.91 aA	0.86 ± 0.60 cB	1.86 ± 0.50bAB	< 0.4 cA
10	2.54 ± 0.78aAB	3.02 ± 0.58 aA	1.58 ± 0.55bAB	1.62 ± 0.51bAB	< 0.4 cA
15	3.10 ± 0.85 aA	2.39 ± 0.45abAB	1.55 ± 0.97bcAB	1.48 ± 0.27 cB	< 0.4 dA

[a] Numbers are mean ± SD ($n = 8$). The lower-case letters after numbers are the significant difference over the row, while the upper-case letters are the significant difference over the column.

Table 4a
Color changes of cherry tomatoes during storage at 21 °C with or without prior washing.*

Treatment	Color parameter	Storage time (d)				
		0	2	5	10	15
Control	L^*	35.66 ± 1.48^{abcd}	35.55 ± 1.46^{abcd}	30.91 ± 2.40^{ef}	31.55 ± 1.85^{def}	29.83 ± 2.30^{f}
	a^*	33.18 ± 1.23^{defg}	33.07 ± 2.13^{efg}	32.16 ± 2.86^{fg}	34.34 ± 2.41^{abcdefg}	31.94 ± 2.89^{g}
	b^*	25.68 ± 1.69^{fg}	25.78 ± 2.30^{efg}	26.94 ± 3.04^{cdefg}	30.55 ± 2.45^{abcd}	28.42 ± 2.32^{abcdefg}
	ΔE^*	–	2.86 ± 1.45^{gh}	6.46 ± 1.83^{abcdefg}	7.25 ± 1.55 ^{abcde}	7.39 ± 2.27 ^{abcd}
NaB	L^*	37.94 ± 1.98^{a}	37.43 ± 2.36^{abc}	36.24 ± 2.84^{abc}	37.15 ± 1.98^{abc}	35.82 ± 2.29^{abcd}
	a^*	33.70 ± 1.64^{cdefg}	33.38 ± 2.55^{bcdefg}	36.49 ± 2.04^{abcde}	37.34 ± 1.61^{abcd}	36.20 ± 2.12^{abcdef}
	b^*	25.65 ± 2.61^{fg}	25.82 ± 1.04 ^{efg}	29.88 ± 2.66^{abcdef}	30.91 ± 1.59^{abc}	31.23 ± 1.34^{abc}
	ΔE^*	3.98 ± 1.00^{cdefgh}	3.43 ± 1.86^{efgh}	6.49 ± 1.88 ^{abcdefg}	7.09 ± 2.21 ^{abcdef}	6.92 ± 1.40 ^{abcdef}
Chlorine	L^*	38.00 ± 1.43^{a}	37.99 ± 1.04^{a}	36.05 ± 1.14^{abcd}	37.92 ± 1.29^{a}	36.44 ± 1.30^{abc}
	a^*	35.29 ± 0.56^{abcdefg}	36.01 ± 1.08^{abcdefg}	38.07 ± 1.27^{ab}	38.26 ± 1.18^{a}	37.55 ± 1.39^{abc}
	b^*	26.42 ± 1.20^{defg}	27.52 ± 1.07^{bcdefg}	32.76 ± 1.23^{a}	31.00 ± 1.00^{abc}	30.59 ± 1.32^{abcd}
	ΔE^*	3.64 ± 0.69^{defgh}	4.38 ± 0.74b^{cdefgh}	8.70 ± 1.54^{a}	7.84 ± 1.15 ^{abc}	6.76 ± 1.72 ^{abcdef}
W2.0	L^*	37.61 ± 1.67^{ab}	37.57 ± 1.86^{ab}	35.39 ± 2.68^{abcde}	37.21 ± 1.66^{abc}	33.25 ± 1.61^{bcdef}
	a^*	34.54 ± 2.49^{abcdefg}	34.37 ± 1.20^{abcdefg}	35.97 ± 1.89^{abcdefg}	38.00 ± 2.50^{ab}	35.22 ± 1.69^{abcdefg}
	b^*	26.06 ± 3.29^{efg}	25.18 ± 1.54^{g}	29.01 ± 2.11^{abcdefg}	31.36 ± 3.43^{ab}	30.19 ± 2.97^{abcde}
	ΔE^*	4.25 ± 2.26 ^{bcdefgh}	3.31 ± 0.72^{fgh}	5.24 ± 2.20 ^{abcdefgh}	7.80 ± 2.80 ^{abc}	7.00 ± 1.29 ^{abcdef}
Neutral water	L^*	34.18 ± 4.56^{abcdef}	36.14 ± 0.91^{abc}	32.90 ± 2.97^{cdef}	35.26 ± 2.15^{abcde}	33.28 ± 1.99^{bcdef}
	a^*	32.14 ± 2.39^{fg}	34.05 ± 1.60^{abcdefg}	33.05 ± 2.02^{efg}	36.48 ± 1.94^{abcde}	35.90 ± 1.86^{abcdefg}
	b^*	25.10 ± 1.62^{g}	25.97 ± 0.82^{efg}	27.69 ± 1.50^{bcdefg}	30.95 ± 1.66^{abc}	32.47 ± 1.71^{a}
	ΔE^*	5.09 ± 1.56 ^{abcdefgh}	1.84 ± 1.14^{h}	4.72 ± 1.53 ^{bcdefgh}	6.66 ± 2.34 ^{abcdefg}	8.03 ± 1.93 ^{ab}

*Washing treatment notations: control – unwashed; NaB – 3000 ppm sodium benzoate at pH 2.0; Chlorine - 200 ppm free chlorine at pH 6.5; W2.0 - distilled water adjusted to pH 2.0. Numbers are mean ± SD ($n = 6$). Mean values with different superscript letters in the same row are significantly different ($P < 0.05$).

Table 4b
Color changes of cherry tomatoes during storage at 4 °C with or without prior washing.*

Treatment	Color parameter	Storage time (d)				
		0	2	5	10	15
Control	L^*	34.64 ± 2.65^{cd}	36.08 ± 0.91^{bcd}	33.99 ± 1.84^{d}	36.09 ± 1.43^{bcd}	34.56 ± 1.56^{d}
	a^*	32.00 ± 2.46^{d}	33.82 ± 1.06^{abcd}	32.81 ± 1.72^{cd}	34.99 ± 1.39^{abcd}	34.42 ± 1.53^{abcd}
	b^*	25.75 ± 2.49^{ab}	26.25 ± 1.94^{ab}	26.72 ± 1.97^{ab}	27.75 ± 1.68^{ab}	26.99 ± 2.14^{ab}
	ΔE^*	–	2.99 ± 1.33^{b}	3.12 ± 0.96^{b}	3.18 ± 1.96 ^{b}	3.31 ± 1.24 ^{b}
NaB	L^*	36.95 ± 3.49^{abcd}	36.22 ± 3.26^{bcd}	37.76 ± 2.99^{abcd}	38.04 ± 3.69^{abcd}	36.80 ± 4.41^{abcd}
	a^*	33.59 ± 1.70^{abcd}	33.41 ± 0.82^{abcd}	34.54 ± 1.47^{abcd}	35.00 ± 1.82^{abcd}	34.99 ± 2.59^{abcd}
	b^*	26.25 ± 2.07 ^{ab}	23.92 ± 2.69^{ab}	26.46 ± 2.31^{ab}	27.48 ± 2.23^{ab}	27.53 ± 2.21^{ab}
	ΔE^*	4.36 ± 2.52^{ab}	4.55 ± 1.78^{ab}	5.17 ± 2.10 ^{ab}	5.00 ± 2.59 ^{ab}	5.29 ± 2.65 ^{ab}
Chlorine	L^*	39.46 ± 1.72^{abc}	38.38 ± 2.00^{abcd}	38.49 ± 2.34^{abcd}	41.27 ± 1.67^{a}	40.49 ± 1.43^{ab}
	a^*	34.47 ± 1.09^{abcd}	33.71 ± 1.47^{abcd}	35.68 ± 1.65^{abcd}	36.89 ± 1.98^{a}	36.11 ± 1.89^{abc}
	b^*	27.28 ± 3.39^{ab}	24.89 ± 3.08^{ab}	29.34 ± 3.46^{a}	29.17 ± 4.52^{ab}	29.06 ± 4.91^{ab}
	ΔE^*	6.33 ± 2.38^{ab}	5.41 ± 1.32^{ab}	7.19 ± 2.78 ^{ab}	8.44 ± 3.52 ^{a}	7.50 ± 3.76 ^{ab}
W2.0	L^*	38.19 ± 1.04^{abcd}	37.87 ± 0.95^{abcd}	37.18 ± 1.75^{abcd}	40.23 ± 1.74^{ab}	38.48 ± 1.47^{abcd}
	a^*	33.04 ± 1.80^{bcd}	32.95 ± 1.30^{cd}	34.60 ± 1.35^{abcd}	35.70 ± 0.69^{abcd}	35.52 ± 1.07^{abcd}
	b^*	24.59 ± 2.24^{ab}	23.39 ± 1.51^{b}	27.36 ± 1.49^{ab}	27.81 ± 4.54^{ab}	26.47 ± 0.89^{ab}
	ΔE^*	4.58 ± 1.50^{abcdef}	4.48 ± 1.04 ^{ab}	4.28 ± 2.02 ^{ab}	6.86 ± 2.68 ^{ab}	3.86 ± 1.77 ^{ab}
Neutral water	L^*	37.82 ± 1.81^{abcd}	38.79 ± 0.67^{abcd}	35.11 ± 3.49^{cd}	40.78 ± 1.11^{ab}	39.47 ± 1.28^{abc}
	a^*	34.28 ± 1.81^{abcd}	33.53 ± 2.08^{abcd}	33.66 ± 1.88^{abcd}	36.77 ± 2.19^{ab}	35.76 ± 2.52^{abc}
	b^*	27.72 ± 1.73^{ab}	24.61 ± 1.59^{ab}	27.60 ± 1.91^{ab}	28.96 ± 2.19^{ab}	27.52 ± 3.30^{ab}
	ΔE^*	4.92 ± 2.15^{abcdef}	5.12 ± 0.98 ^{abcdef}	4.36 ± 2.04 ^{ab}	7.39 ± 1.91 ^{ab}	6.01 ± 2.24 ^{ab}

*Washing treatment notations: control – unwashed; NaB – 3000 ppm sodium benzoate at pH 2.0; Chlorine - 200 ppm free chlorine at pH 6.5; W2.0 - distilled water adjusted to pH 2.0. Numbers are mean ± SD ($n = 6$). Mean values with different superscript letters in the same row are significantly different ($P < 0.05$).

For the chlorine treatment, Gram-positive *L. monocytogenes* was far more sensitive than the two Gram-negative bacteria. The free chlorine treatment reduced the population of *L. monocytogenes* by ~5 log CFU/g after 3-min rinsing, while the reduction of *S. enterica* and *E. coli* O157:H7 was only ~1–2 log CFU/g. The population of *L. monocytogenes* decreased to a level close to the detection limit after 5-d storage at 4 °C and 10-day storage at RT. The respective population of *E. coli* O157:H7 was 1.90 log CFU/g and 0.63 log CFU/g after 15 d storage at 4 and 21 °C, and that of *S. enterica* was 4.98 log CFU/g and 3.88 log CFU/g after 15 d storage at 4 °C and 21 °C, respectively. Our results are different from similar reductions of the three pathogens on leafy greens caused by 50 ppm free chlorine at pH 6.5, as reported by Stopforth et al. (2008). The difference may result from variations in surface properties of different produce commodities and the subsequent attachment properties of bacteria (Wang et al., 2012).

Acidified NaB resulted in an initial reduction of > 4 log CFU/g against the three tested pathogens after 3-min washing, followed by further reduction to an extent depending on bacterial strain and storage temperature. For *E. coli* O157:H7, the survival bacterial population decreased to values below the detection level during storage at day 5 and day 10 at 4 °C and RT, respectively. The reduction of *S. enterica* to a value below the detection limit during storage was observed on day 15 at 4 °C, but 2–2.5 log CFU/g was detected after 5 d storage at RT (Tables 1 and 2). For *L. monocytogenes*, > 1 log CFU/g was detected during storage at both 4 °C and RT, and there was no significant difference ($P > 0.05$) from day 2 to day 15. It has been reported that the

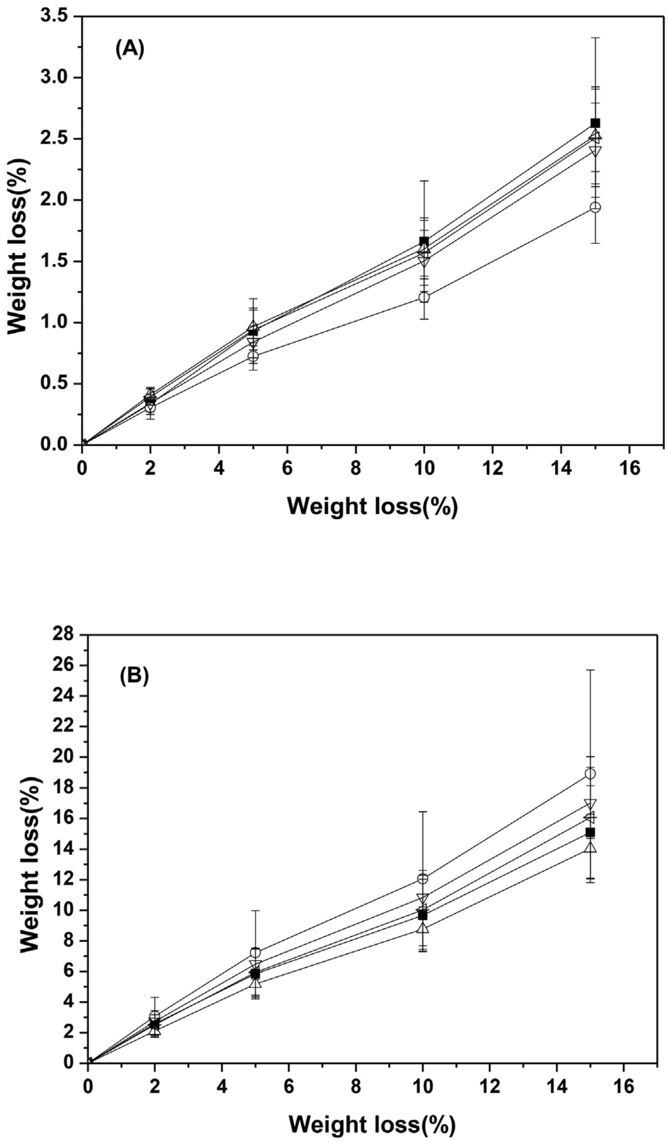

Fig. 1. Changes in weight loss (%) of tomatoes stored at 4 °C (A) and room temperature of ~21 °C (B) after different washing treatments. Symbol notations: (■) 3000 ppm sodium benzoate at pH 2.0, (◯) 200 ppm free chlorine at pH 6.5, (△) distilled water adjusted to pH 2.0, (▽) neutral distilled water, (◁) control. Error bars are SD (n = 5).

combination of low pH with antimicrobials can enhance the antimicrobial activity. Patil et al. (2010) reported that the time required for 5 log reduction of *E. coli* in apple juice decreased as the pH decreased from 5.0 to 3.0. In another recent study, the reduction of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in apple juice by ozone was 3 log CFU/mL higher when juice pH was adjusted to 3.0 than 5.0, while acidity alone did not change the bacterial population significantly (Song et al., 2015). Overall, 3000 ppm NaB acidified to pH 2.0 was the most effective treatment at the studied conditions.

3.2. Background microbial loads of cherry tomatoes during storage

Changes in total yeast and mold counts (TYMC) on tomatoes after washing treatment and during storage at 4 °C and RT are presented in Table 3. The TYMC on the untreated control tomatoes was about 3.0–4.0 log CFU/g, which is in line with the findings of Mukhopadhyay et al. (2014) but is higher than the ~2.0 log CFU/g on untreated Roma tomato cubes reported by Schmidt et al. (2006). This was expected due to differences in tomato cultivar and sample preparation. We also observed the variation of TYMC from different batches of tomatoes bought from the same supermarket (Table 4a vs 4b, control groups).

During storage, the TYMC of the unwashed control group kept growing, reaching the highest value after 15 d (Table 3). After washing, the TYMC was reduced, with an extent dependent on the washing solution formulations. Except for the NaB treatment, the TYMC was generally above 1.5 log CFU/g and was similar between each other ($P > 0.05$). The NaB treatments resulted in the TYMC range from 0.57 to 1.12 log CFU/g ($P > 0.05$) at 4 °C (Table 3a) and always below the detection limit at RT (Table 3b). This was expected due to the well-known function of NaB as a preservative inhibiting fungi to prevent spoilage (Chipley, 2005). Free chlorine is not effective in controlling molds and yeasts (Joshi et al., 2013), and acidified electrolyzed water reduced molds by 2 log CFU/g on lettuce (Koseki et al., 2001). The findings in Table 3 indicated the potential of acidified NaB washing to extend the shelf-life of fresh produce.

3.3. Quality changes of cherry tomatoes during storage

3.3.1. Color

The impacts of washing treatments on tomato color were quantified by Hunter L^*, a^* and b^* parameters. Results are summarized in Tables 4a and 4b for storage treatments at RT and 4 °C, respectively. At RT (Table 4a), L^*, a^*, and b^* values of each group decreased, increased, and increased, respectively, indicating the darkening and ripening of tomatoes during storage (Fagundes et al., 2015; Mukhopadhyay et al., 2014). However, these changes were not significant ($P > 0.05$) at 4 °C (Table 4b).

In order to further understand color differences between treatments,

Table 5
Changes in firmness (N) of cherry tomatoes during storage at 4 and 21 °C with or without prior washing.[a]

Temp.	Treatment	Storage time (d)				
		0	2	5	10	15
4 °C	Control	8.68 ± 1.00^{abcd}	7.97 ± 0.97^{abcd}	8.90 ± 1.01^{abcd}	7.48 ± 0.77^{abcdefg}	8.10 ± 1.37^{abcd}
	NaB	–	9.17 ± 2.14^{abcd}	8.96 ± 0.71^{abcd}	8.53 ± 0.90^{abcd}	9.01 ± 1.71^{abcd}
	Chlorine	–	8.14 ± 2.16^{abcd}	7.87 ± 0.56^{abcdef}	8.14 ± 0.56^{abcd}	8.01 ± 1.21^{abcd}
	W2.0	–	9.40 ± 1.26^{ab}	9.88 ± 0.78^{a}	7.94 ± 0.59^{abcd}	8.64 ± 1.55^{abcd}
	Neutral water	–	9.02 ± 1.06^{abcd}	9.12 ± 1.28^{abcd}	8.03 ± 0.92^{abcd}	8.07 ± 0.84^{abcd}
21 °C	Control	–	7.85 ± 1.92^{abcdef}	8.34 ± 0.91^{abcd}	6.35 ± 0.32^{defgh}	4.55 ± 0.45^{g}
	NaB	–	9.41 ± 1.48^{ab}	8.97 ± 1.44^{abcd}	6.62 ± 0.72^{bcdefgh}	5.09 ± 0.93^{fgh}
	Chlorine	–	8.51 ± 0.84^{abcd}	9.20 ± 1.40^{abcd}	6.68 ± 0.58^{bcdefgh}	5.06 ± 0.58^{fgh}
	W2.0	–	8.80 ± 1.15^{abcde}	8.99 ± 0.94^{abcd}	6.60 ± 0.53^{defgh}	4.86 ± 0.71^{gh}
	Neutral water	–	7.89 ± 1.13^{abcde}	7.96 ± 1.55^{abcd}	6.52 ± 0.49^{cdefgh}	4.45 ± 0.28^{h}

[a] Washing treatment notations: control – unwashed; NaB – 3000 ppm sodium benzoate at pH 2.0; Chlorine - 200 ppm free chlorine at pH 6.5; W2.0 - distilled water adjusted to pH 2.0. Numbers are mean ± SD (n = 5). Mean values with different superscript letters are significantly different ($P < 0.05$).

ΔE^* (eq. (1)) values were calculated (Tables 4a and b). According to Kim et al. (2008), a ΔE^* value of 0–0.5 indicates an imperceptible difference in color, 0.5–1.5 a slight difference, 1.5–3.0 a just noticeable difference, 3.0–6.0 a marked difference, 6.0–12.0 an extremely marked difference, and > 12.0 a color of a different shade. Therefore, tomatoes washed with acidified NaB had a marked difference from the control over storage time at 4 °C, while free chlorine treatment caused an extremely marked difference. Based on ΔE^*, it can be further speculated, that tomatoes washed with acidified NaB had a lower ripening rate than those treated with free chlorine.

3.3.2. Total soluble solids (TSS) content

The TSS content of tomatoes is used as an indicator of tomato quality since it may suggest the sweetness of tomatoes (Pataro et al., 2015). The TSS contents of all untreated and treated tomatoes remained constant (data not shown, $P > 0.05$) during 15-d storage at RT and 4 °C. Similar results were reported by Maedeh (2012) that the TSS content of tomatoes was not significantly different when stored at different temperatures. Therefore, the acidified NaB wash did not change the sweetness.

3.3.3. Weight loss

Weight loss% of washed tomatoes during storage is shown in Fig. 1. As expected, weight loss increased over time for all treatments at 4 °C and RT. After 15 d storage at 4 °C, the weight loss% ranged from 1.94% to 2.63%, and no significant difference was found among these treatments. The weight loss% of cherry tomatoes stored at RT exhibited a pattern similar to that stored at 4 °C but had a higher magnitude of 14.05–18.91%. The higher weight loss% at RT than at 4 °C can be attributed to the faster respiration and transpiration (Javanmardi and Kubota, 2006) and the open vs. closed (in a refrigerator) environments.

3.3.4. Firmness

The firmness of tomatoes was not affected ($P > 0.05$) by the washing solutions during post-washing storage (Table 5). Over the duration of storage, the firmness of control fruits did not change significantly at 4 °C, while the reduction was very significant at RT. Fruit softening is a biochemical process with pectin in the cell wall being hydrolyzed by enzymes such as pectinesterase and polygalacturonase (Brecht et al., 2007; Fagundes et al., 2014), and the rate of enzymatic reactions is expected to be higher at RT than at 4 °C. In addition, reduction of firmness during storage was found to be positively correlated to the weight loss of blueberries (Paniagua et al., 2013) and tomatoes (Colgecen and Aday, 2015). Data in Table 5 and Fig. 1 seem to agree with this correlation. The weight loss data at 4 °C in Fig. 1 are similar to the study of Mukhopadhyay et al. (2014) but different from the reduction of firmness during refrigerated storage in a few other studies (Choi et al., 2015; Fagundes et al., 2015), which can be due to differences in tomato cultivar and maturity used in different studies.

4. Conclusion

The present study demonstrated that acidified NaB can be used to wash post-harvest tomatoes to decontaminate pathogens. By rinsing tomatoes with 3000 ppm NaB acidified to pH 2.0 for 3 min, 4–5 log CFU/g reductions were achieved for *S. enterica*, *E. coli* O157:H7 and *L. monocytogenes* inoculated at a high level on tomatoes. In comparison, 200 ppm free chlorine was effective against *L. monocytogenes* but less effective against *S. enterica* and *E. coli* O157:H7. Additionally, acidified NaB effectively reduced the background yeasts and molds but did not change the color, weight loss, TSS content, and firmness of tomatoes when compared to the control groups. These findings, together with its regulatory status and low-cost, suggest the potential of highly acidified NaB as an alternative post-harvest washing solution of fresh produce.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.01.013.

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