

Research Note

Efficacy of Electrolyzed Water and an Acidic Formulation Compared with Regularly Used Chemical Sanitizers for Tableware Sanitization during Mechanical and Manual Ware-Washing Protocols

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ABSTRACT

This study investigated residual bacteria and different food types left on tableware items after various washing and sanitization protocols. *Escherichia coli* K-12 and *Staphylococcus epidermidis* were inoculated into whole milk and soft cream cheese. The milk was used to contaminate regular drinking glasses and the cheese was used to contaminate plates and silverware. These tableware items were washed in manual (43°C) and mechanical (49°C) washers and sanitized with different sanitizers (24°C) for 5 s. Quaternary ammonium compound, sodium hypochlorite, peroxyacetic acid, neutral electrolyzed water (NEW), and a combination of citric acid with sodium dodecylbenzene sulfonate (acidic formulation) were used as the chemical sanitizers. Tap water was used as a control. Results showed that at least 5-log reductions in both bacterial numbers were achieved for all sanitizers in both types of washers, except for the control. With mechanical dishwashing, the NEW and acidic formulation treatments reduced bacterial populations by >6.9 and >6.0 log CFU per tableware item, respectively. With the manual operation, bacterial numbers were reduced by >5.4 and >6.0 log CFU per tableware item, respectively. This study revealed that NEW and the acidic formulation are as effective as the other chemical sanitizers for food contact surface sanitization in manual and mechanical ware washing.

Cross-contamination with pathogenic bacteria can result from food processing and preparation equipment and tableware that are not properly cleaned and sanitized. Contamination of food contact surfaces was a major problem mentioned in a recent U.S. Food and Drug Administration (FDA) report (19). Thus, cleaning and sanitization of these items should be optimized. The FDA *Food Code* (20) defines *sanitization* as the application of cumulative heat or chemicals on food contact surfaces that, when evaluated for efficiency, is sufficient to yield a 5-log reduction in representative pathogenic microorganisms. In order to achieve this standard for eating and food preparation utensils, many restaurants utilize manual, semiautomated, or fully automated dish washing systems, or a combination of these. The washing protocols for automatic dishwashers using only hot water or a water-chemical combination are reported in documents prepared by NSF International (14). The protocol for manual ware washing can be found in the *Food Code*. Both documents mandate that a 5-log reduction in bacterial species inoculated onto eating utensils should be achieved at an appropriate sanitizer-detergent concentration, temperature, and time combination. In addition to this, the docu-

ments also require that all “old food” be removed from the contact surfaces after the washing protocol.

Sanitizing agents most widely used in ware-washing operations include chlorine, iodine, and quaternary ammonium compounds (QAC). Used at the correct concentration-time-temperature combination on utensils with moderate organic matter and bacterial loads, these sanitizers have been shown to have the ability to reduce bacterial numbers to acceptable levels. Other agents showing the ability to significantly reduce bacterial numbers on food contact surfaces include ionizing radiation (including UV light), ozone, electrolyzed water, and acidic sanitizers. All new sanitizers that will be used in ware-washing operations should be evaluated to ensure that they meet the minimum requirements as set forth in the FDA and the NSF mandates. Two potential, but untested, sanitizers for automatic and manual ware-washing operations are neutral electrolyzed water (NEW) and an acidic formulation (a food-grade organic acidic sanitizer made from natural products). Advantages of these sanitizers are that they will not have residual potential, and they will not irritate the skin nor will they be corrosive to the equipment. Indeed, in U.S. Environmental Protection Agency documents, the agency expresses concerns about high levels of residual halogenated compounds that could bioaccumulate in the environment (18).

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Previous studies have shown that NEW is effective in reducing microbial numbers on freshly cut vegetables such as spinach, tomato, and lettuce (2, 5, 8), and in infected root canals in dentistry (4). The acidic formulation is also effective in reducing bacterial numbers on plastic and metal surfaces and on disposable fabric wipes (10, 11). To ensure a proper evaluation of the NEW and the acidic formulation solutions, they were tested in mechanical and manual washing operations and against *Escherichia coli* K-12 (gram negative) and *Staphylococcus epidermidis* (gram positive) microorganisms inoculated onto ceramic plates, stainless steel cutleries (forks, spoons, knives), and drinking glasses. The objective of this study was to compare the sanitization efficiency of NEW and the acidic formulation with other regularly used formulations for reduction of selected bacteria on tableware items in manual and mechanical washing protocols. The tableware items would be soiled with inoculated milk and soft cream cheese.

MATERIALS AND METHODS

Bacterial cultures. *E. coli* K-12 (ATCC 29181) and *S. epidermidis* (ATCC 12228) were used in this study. Prior to testing, cultures of the *E. coli* K-12 and *S. epidermidis* were stored frozen (-80°C) in 30% (vol/vol) sterile glycerol (Fisher Scientific, Fair Lawn, NJ). The stock culture was prepared by transferring a loopful of the *E. coli* and *S. epidermidis* into 50 ml of Trypticase soy broth (Difco, Becton Dickinson, Sparks, MD) containing 0.3% (wt/wt) yeast extract (Fisher Scientific) (TSBYE) and then incubating the cultures at 37°C for 18 h. A loopful of this broth was then inoculated into Trypticase soy agar (Difco, Becton Dickinson) supplemented with 0.3% (wt/wt) yeast extract (TSAYE) and incubated at 37°C for 24 h. This TSAYE containing the cell cultures was stored in a refrigerator at 3°C and used as a stock culture.

Prior to each experiment, a loopful of each of the stock cultures of *E. coli* K-12 and *S. epidermidis* was propagated aerobically in 50 ml of TSBYE at 37°C for 18 h. A 35-ml aliquot of each cell broth was centrifuged (Kendro Laboratory Products, Sorvall RC 5C Plus, Newtown, CT) at $10,000 \times g$ for 10 min at 4°C . The supernatant fluid was decanted and the cell suspension resuspended in 35 ml of sterile 0.1 M potassium phosphate buffer (pH 7.2) until an initial concentration of approximately 10^{10} CFU/ml for *E. coli* K-12 or 10^9 CFU/ml for *S. epidermidis* was achieved. Each cell suspension was separately mixed with the food samples to be tested.

Food sample preparation. Aseptically processed soft cream cheese and pasteurized whole milk were used as the food contact surface contaminants. The food samples were purchased from a local grocery store 1 day prior to the experiment. They were both stored in a refrigerator at $4 \pm 1^{\circ}\text{C}$. When ready for use, a 90-g portion of the soft cream cheese or a 180-g aliquot of the milk was weighed into a beaker with a magnetic stirring bar. Each food sample was then inoculated with the cell suspensions of *E. coli* or *S. epidermidis* (1:10 [wt/wt]) and mixed for about 5 min.

The ceramic plates, stainless steel forks, spoons, knives, and drinking glasses were sterilized by autoclaving at 121°C for 15 min before each experiment. The weights of the soft cream cheese pasted onto the tableware items were as follows: plates, 5 g; forks, 0.5 g; spoons, 0.5 g; and knives, 0.5 g. The glasses were contaminated with inoculated whole milk (0.5 g). The contaminated cream cheese was applied to the entire upper surface of the plates and the top half of the spoons, forks, and knives. Contaminated

milk was applied to the inner wall of the drinking glasses. Eight replicates were prepared. The tableware items were air dried for 1 h at $24 \pm 2^{\circ}\text{C}$ on a flat, sterile surface prior to washing. Before and after the air-drying procedure, each tableware item type was sampled, and the bacterial survival numbers were determined by the plate count method.

Preparation of sanitizer solutions. An electrolyzed water generator (Stel-80) manufactured by Trustwater, Ltd. (Tipperary, Ireland), was used to produce the NEW solution. The NEW solution was generated from a saturated sodium chloride (Morton International, Inc., Chicago, IL) solution at an instrument setting of 3.1 to 3.2 A, 19.6 to 21.0 V, and a water flow rate of 75.7 liters/h. The water that was generated contained approximately 100 ppm of free available chlorine, with a pH of 7.4 ± 0.2 . The NEW was made immediately prior to the experiment. The free available chlorine concentration was measured with a HI 95771 Chlorine Ultra High Range Meter (Hanna Instruments, Ann Arbor, MI). The oxidation-reduction potential value was measured with a Titrators Model DL70ES tester (Mettler Toledo, Columbus, OH).

The other sanitizers that were tested included 10,000 ppm of the acidic formulation (an organic acid sanitizer containing citric acid and sodium dodecylbenzene sulfonate; Microcide, Inc., Troy, MI), 100 ppm of sodium hypochlorite (Chlor-Clean 12.5, Madison Chemical Co., Inc., Madison, IN), 200 ppm of QAC (Ster-Bac, Klenzade, Division of Ecolab, Inc., St. Paul, MN), and 1,000 ppm of peroxyacetic acid (Oxywave, Madison Chemical Co., Inc.). These concentrations were recommended by the respective manufacturers. The FDA *Food Code* (2005) states that the manufacturers' recommended concentrations should be used for detergents and sanitizers in washing protocols (20).

Mechanical ware washing of the test tableware. The mechanical dishwasher used in this study was an AM Select Door-Style washer manufactured by Hobart, Inc. (Troy, OH). All tableware items were washed with 1,000 ppm of Guardian Score detergent (Ecolab, Inc.) at 49°C and sanitized with the different types of sanitizers at 24°C . Each day, before using the dishwasher, it was cleaned with hot water and refilled with a fresh lot of the detergent solution. The sanitizing solutions were injected into the final rinse manifold of the equipment during the rinse cycle as recommended by the manufacturer.

At the beginning of each cleaning operation, the contaminated tableware items were placed in a sterilized rack that was then positioned inside the dishwasher. The washing and sanitizing waters were sprayed onto the tableware items from both top and bottom slotted pipes within the washer. The pressure of the sanitizing water was 138 kPa. During the washing step, the detergent solution was sprayed onto the dishes for 40 s. The tableware items were then sprayed with the selected sanitizer for 10 s. After this sanitization cycle, all tableware items were air dried for 1 h at $24 \pm 2^{\circ}\text{C}$ on a sterile surface prior to sampling.

Manual ware washing of the test tableware. The manual dishwasher was made of three compartments: washing, rinsing, and sanitizing. The wash sink held approximately 227 liters of the detergent solution, while the rinse and sanitizer sinks each contained approximately 150 liters of solution. The tableware items were washed with 100 ppm of Mag Fusion detergent (Ecolab, Inc.) at 43°C for 20 s, rinsed with tap water at 24°C for 5 s, and finally sanitized with each of the different sanitizers at 24°C for 5 s. Before each test, the dishwasher was cleaned with hot water and refilled with fresh water and detergent-sanitizer.

During the washing step, each contaminated tableware item

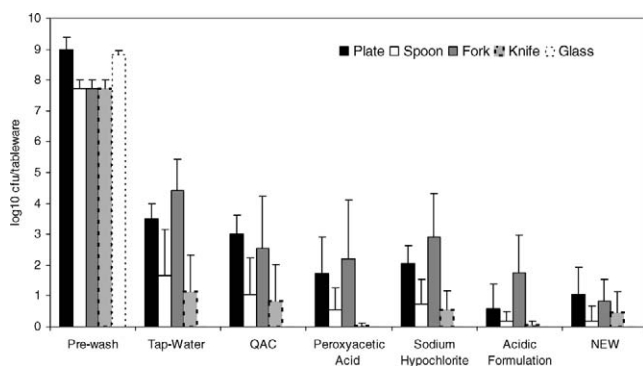


FIGURE 1. Enumeration of *E. coli* K-12 on tableware before and after washing and sanitizing, using the mechanical dishwasher.

was washed manually (wearing rubber gloves) by using a Scotch-Brite multipurpose scrub sponge (3M, St. Paul, MN) attached to a scrubbing device. This was spring loaded, and it allowed us to apply an average force of 0.8 ± 0.02 kg to each tableware item. The plates and glasses were washed by using four clockwise and four counterclockwise strokes. The spoons, forks, and knives were washed by using two forward and two backward strokes with the scrubbing device. During the washing, rinsing, and sanitizing, all the tableware items were completely immersed in the solutions. The items were placed on a sterile rack after the sanitization step, and then they were air dried for 1 h at $24 \pm 2^\circ\text{C}$ before microbial testing.

Procedures for microbial enumeration of the contaminated tableware surfaces. Enumeration of the microorganisms on each tableware item began by using hygienic cotton swabs to transfer the organisms from the surface of each plate, cutlery, and drinking glass to test tubes containing 2 ml of maximum recovery diluent (Oxoid, Ltd., Basingstoke, Hampshire, England). Before sampling the tableware items, the swabs were moistened with the sterile maximum recovery diluent solution. Each swab was used to remove the inoculated microorganisms and then to transfer them to the test tubes, in which they were vortexed vigorously to remove bacteria from the fiber tip.

For enumeration of the total viable counts, the contents of the test tubes were serially diluted and plated into TSAYE. The plates were incubated at 37°C for 36 h. The bacteria cells were counted with a Darkfield colony counter (American Optical, Buffalo, NY). The detection limit for estimating the bacterial numbers was 20 CFU per utensils for the plates and forks, and 2 CFU per utensils for the spoons, knives, and glasses.

Statistical analysis. All trials were repeated at least eight times in this study. Microbial counts were expressed as log CFU per milliliter (inoculum) and log CFU per tableware item (surface). The reported values of the total plate counts were the mean values of eight trials \pm standard deviations. The data analyses were performed by analysis of variance and Tukey's test, with the SPSS, version 16.0, statistical program (SPSS, Inc., Chicago, IL) to determine the level of significance between the effect of each sanitizer, washing type, bacterial species, and tableware item. A *P* value of 0.05 was set for the level of significance.

RESULTS AND DISCUSSION

Reduction in bacterial counts during air drying of the different tableware items. The reductions in bacterial counts for *E. coli* and *S. epidermidis* inoculated onto the tableware items before and after the 1-h drying were ex-

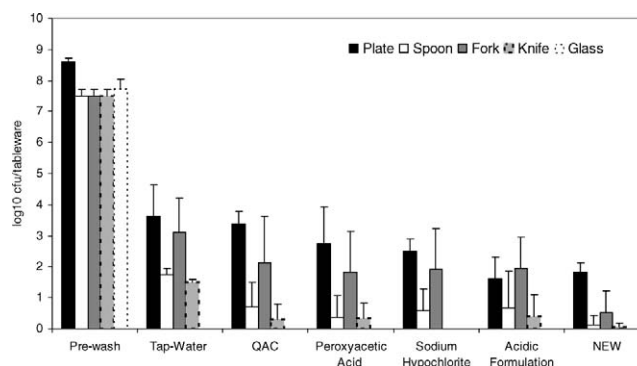


FIGURE 2. Enumeration of *S. epidermidis* on tableware before and after washing and sanitizing, using the mechanical dishwasher.

amined. The mean reduction in the population of *E. coli* following this drying at $24 \pm 2^\circ\text{C}$ ranged between 0.3 and 0.5 log CFU per tableware item. For *S. epidermidis*, the reduction was 0.2 to 0.3 log CFU per tableware item. This showed that a large percentage of the bacteria inoculated onto the tableware items survived during the 1-h drying. In support of this, the statistical analysis showed that there were no significant ($P > 0.05$) differences between the bacterial populations before and after drying of the inoculated food products pasted onto the tableware items. These findings are similar to those from previous studies reported by Lee et al. (9) for *E. coli* K-12 and *Listeria innocua* inoculated onto various utensils then allowed a 1-h drying time. A comparison of the survival patterns for these two organisms showed that the desiccation stability of *E. coli* and *S. epidermidis* were similar. The tableware items were air dried at room temperature in order to simulate normal washing procedures in food preparation establishments and to provide enough contact time for the food contaminants to adhere to the tableware items (13).

Efficacy of the mechanical ware washing, using various sanitizing agents. To test the efficiency of chemical sanitizers for food contact surfaces, the U.S. Environmental Protection Agency recommends the use of *E. coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC 6538) as test organisms (17). To comply with this recommendation, this study used nonpathogenic *E. coli* K-12 and *S. epidermidis* as surrogates for those standard test pathogenic bacteria (7). The tableware items and the food products were both tested to ensure that they had no *E. coli* or *S. epidermidis* prior to the experiment. The tableware items were also visually clean before they were washed as a part of the test protocol.

Figures 1 and 2 show differences in the viable counts between the initial levels of *E. coli* K-12 and *S. epidermidis* on the tableware (prewash) and after treatment with the sanitizers. Statistically, there were significant ($P < 0.05$) differences in the bacterial reductions between the treatment with tap water compared with most of the other sanitizers for many of the tableware items. The viable *E. coli* cells left on the plates, spoons, forks, and knives after washing at 49°C and sanitizing at 24°C with tap water were 3.5, 1.6, 4.4, and 1.1 log CFU per tableware item, respectively (Fig.

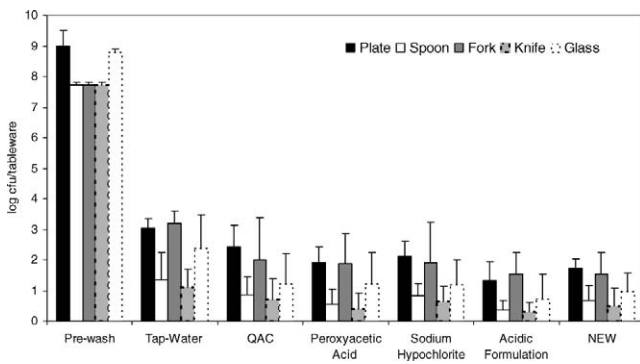


FIGURE 3. Enumeration of *E. coli* K-12 on tableware before and after washing and sanitizing, using the manual dishwasher.

1). The results for *S. epidermidis* were 3.6, 1.7, 3.1, and 1.5 log CFU per tableware respectively, as seen in Figure 2. For the bacterial population left on the glasses, the counts were less than the detection limit of 2 CFU per utensil. The tap water treatment (control) by itself showed some reduction in bacterial count. However, the treatment on the forks did not produce a 5-log bacterial reduction. The FDA *Food Code* and the ANSI/NSF International standards both mandate that any chemical sanitizing treatment used in ware-washing operation should achieve a 5-log microbial reduction. Because of this requirement, it can be deduced that the use of tap water by itself is not a suitable sanitizer for the cleaning of tableware items at the conditions used in this study.

The results from Figures 1 and 2 also show that all the chemical sanitizers were able to produce 5-log CFU reductions of *E. coli* and *S. epidermidis* during mechanical dish-washing. When a comparison is made between each sanitizer treatment, the NEW and the acidic formulation sanitizers were more effective in producing a higher *E. coli* and *S. epidermidis* reduction when compared with the others. The NEW and the acidic formulation sanitizers produced the final bacterial counts of <1.8 log CFU per tableware item for both organisms, respectively. These results show that the use of the NEW and the acidic formulation together with the mechanical ware washing were efficient in eliminating both types of bacteria. Also, there were no significant ($P > 0.05$) differences between the effectiveness of the NEW and the acidic formulation when they were tested on both bacterial types. Despite the fact that these sanitizers were able to produce 5-log bacterial reductions, the QAC and acidic formulation formed filmlike residues on the tableware surfaces after the washing procedures. Thus, another rinsing might be necessary for these types of sanitizers. An investigation into this should form a part of future studies.

This study also observed the effect of mechanical dish-washing on the different types of tableware items that had been contaminated with inoculated food products. The glasses had significantly ($P < 0.05$) fewer microbial numbers when compared with the other tableware items. As seen in Figures 1 and 2, the glasses were the easiest tableware items to be cleaned, since the bacterial load was reduced to undetectable numbers. An explanation for this re-

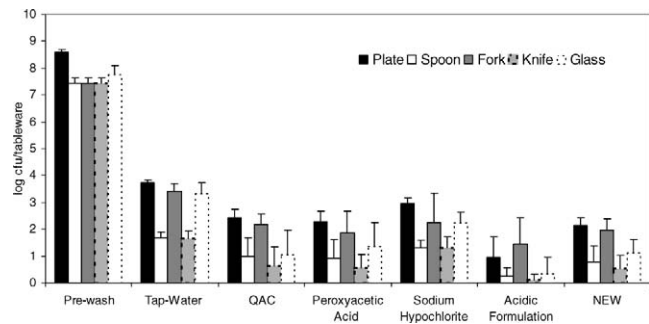


FIGURE 4. Enumeration of *S. epidermidis* on tableware before and after washing and sanitizing, using the manual dishwasher.

sult could be that the water pressure (138 kPa) and temperature in the mechanical ware-washing machine were sufficient to break the adhesion bonds between the glass surface and the contaminated milk products. Further studies with a longer drying time (>1 h) prior to washing might be necessary to see whether the same results would be achieved. The highest microbial counts after the washing procedures were obtained on the plates and forks, which were followed by the knives and spoons. This may have occurred because the plates had the largest surface area of all the tableware items. Figures 1 and 2 showed that this type of tableware had the highest initial bacterial count prior to the washing step. This may have resulted in a higher probability of organic matter being present before the sanitizing process, and this may have helped to shield the bacteria from the maximum effect of the heat and sanitizing agents. In the case of the forks, the results obtained may have occurred because of the difficulty in removing the food contaminants and bacteria lodged in the spaces between the prongs. Because the silverware items in the ware-washing trays within the washer were not always oriented in the same direction and similarly positioned prior to each test, it is possible that they all may not have been uniformly bathed with the washing and sanitizing solutions. Thus, in some cases, the food might have been partially dislodged from the spaces between the prongs of the forks. Higher temperatures and longer contact times during the washing cycles might be required to solve this problem.

Efficacy of the manual ware washing, using various sanitizing agents. During manual ware washing, all tableware items were washed by one individual as a means of minimizing variability in the study. This study also implemented the minimum temperatures required for manual ware-washing protocols as required by the FDA *Food Code*.

Figures 3 and 4 show that the tap water treatment did not completely generate the 5-log bacterial reductions for both bacteria types. These results are similar to the findings obtained for the mechanical ware-washing operation, where the tap water treatment was significantly ($P < 0.05$) least effective for inactivation of both bacterial types on the contaminated tableware items. This confirms that tap water treatment would not be a proper sanitizing agent when used at the temperature selected during this study. It should be noted that the FDA *Food Code* regulations mention that the

minimum temperature for hot water sanitization is 71°C for mechanical and 77°C for manual ware washing for at least 30 s (20).

As was the case with the mechanical ware washing, the use of sodium hypochlorite, QAC, peroxyacetic acid, the acidic formulation, and NEW solutions on both bacterial types, also produced ≥ 5 -log CFU per tableware reductions during the manual ware-washing operation (Figs. 3 and 4). However, published reports have noted that some of these sanitizers can bioaccumulate in the environment and/or be corrosive to equipment, and are not recommended for use in some countries (16, 21). The NEW solution with a neutral pH (7.4 ± 0.2) can be a safe alternative sanitizing agent for various ware-washing operations. The results of the manual ware-washing protocol showed that the NEW solution produced bacterial reductions between 6.2 and 7.8 log CFU per tableware items for *E. coli*, and 5.4 between 6.9 log CFU per tableware item for *S. epidermidis*, respectively. According to studies by Deza et al. (2), NEW has many advantages when compared with other traditional sanitizers. Since NEW sanitizers can be easily produced on-site, they pose less transport and storage logistical problems. They are also less hazardous to workers and to the ecosystem. In addition to these advantages, several researchers have reported that high oxidation-reduction potential (~ 800 mV) and the free available chlorine content (100 ppm) of NEW sanitizers are factors that play critical roles in the inactivating of microorganisms (1, 6, 12, 15). Thus, NEW does not depend on a high or low pH for its bacterial killing action. As a result, NEW should not be corrosive to metal surfaces and this is an advantage. As a future study, different concentrations of free available chlorine associated with NEW treatments could be investigated for the inactivation of various bacterial species.

For *E. coli* as the contaminating organism, the statistical analysis showed that both the acidic formulation and NEW sanitizer were not significantly ($P > 0.05$) better in producing higher bacterial losses when compared with the other sanitizer treatments (Fig. 3). However, the acidic formulation solution performed better in reducing the *S. epidermidis* population, with reductions of 6.0 to 7.7 log CFU per tableware item, when compared with the NEW and other sanitizers (Fig. 4).

In this manual ware-washing study, the microbiological counts on the tableware items were similar to the results obtained for the mechanical ware-washing operation. Statistically, the plates and forks that were contaminated with bacteria showed the highest viable counts after the washing procedures, which were followed by the glasses, spoons, and then the knives. It was also noticed that a small number of the glasses from the manual washing protocol had traces of the food contaminants on them. None of this was seen on the glasses from the mechanical protocol. This might have occurred because of the difficulty in cleaning the curved inner surface area of the glasses, especially where the wall meets the bottom part of the glass (2).

In conclusion, this study demonstrated that NEW and the acidic formulation could be used in an effective protocol for reducing microbial contamination on various ta-

bleware items in mechanical and manual ware-washing operations. These treatments showed that they had the ability to produce (at least) similar or better (in some cases) bacterial reductions when compared with the other traditional sanitizers. Thus, NEW could be used as an alternative sanitization agent for food contact surfaces in kitchens, restaurants, food service, and food processing establishments. The highest bacterial loads remaining on the tableware after the dishwashing operations were on the plates and forks.

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