

1 **Efficacy of Neutral Electrolyzed Water, Quaternary Ammonium and Lactic Acid**
2 **Based Solutions in Controlling Microbial Contamination of Food Cutting Boards**

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14 **Running Title: Efficacy of Neutral Electrolyzed Water, Quaternary Ammonium and Lactic**
15 **Acid Based Solutions in Controlling Microbial Contamination of Food Cutting Boards**

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21 **Abstract**

22 Bactericidal activity of neutral electrolyzed water (NEW), quaternary ammonium (QUAT), and lactic
23 acid based solutions was investigated at ambient temperature against *Salmonella* Typhimurium,
24 *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Listeria monocytogenes* and *Staphylococcus*
25 *aureus* that were inoculated onto the surface of scarred polypropylene and wooden food cutting
26 boards. Antimicrobial activity was also examined when using cutting boards in preparation of raw
27 chopped beef, chicken tenders or salmon fillet. Viable counts of survivors were determined as log
28 CFU/100 cm² within 0 (untreated control), 1, 3 and 5 min of treatment. Within the first minute of
29 treatment, NEW and QUAT solutions caused more than 3 log bacterial reductions on polypropylene
30 surfaces whereas less than 3 log reductions were achieved on wooden surfaces. After 5 min of
31 treatment, more than 5 log reductions were achieved in all bacterial strains inoculated onto
32 polypropylene surfaces. Using NEW and QUAT solutions within 5 min reduced Gram-negative
33 bacteria by 4.58-4.85 log compared to more than 5 log reductions in Gram-positive bacteria
34 inoculated onto wooden surfaces. Lactic acid treatment was significantly less effective ($P < 0.5$)
35 compared to NEW and QUAT treatments. However, a considerable decline in antimicrobial
36 effectiveness was observed when both cutting board types were used to prepared raw meat.

37 **Keywords:** Neutral electrolyzed water, quaternary ammonium, lactic acid, cutting boards.

38 **Practical Application**

39 NEW could be used as an effective alternative to commonly used chemical sanitizers such as QUATS.
40 Treatments effectiveness against microbial contamination was higher on polypropylene compared to
41 wooden surfaces.

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44 **Introduction**

45 Contamination, growth and survival of pathogenic bacteria during food preparation may cause
46 several foodborne outbreaks which may impose significant health and economical threats. During the
47 past decade, increasing industrialization and urban living caused considerable changes in eating
48 habits with increased sales of ready-to-eat meals in which processed food has become more
49 vulnerable to bacterial contamination (Taylor and others 1999; Adams and Motarjemi 1999; Langsrud
50 and others 2003; Moretro and others 2011). Accordingly, any breakdown in food hygiene during
51 meals preparation in restaurants and high-volume food processing facilities may result in more people
52 to be affected, spending millions of dollars due to medical expenses and decrease in employee
53 productivity (Adams and Motarjemi 1999; Monnin and others 2012).

54 The equipment and utensils used in food preparation may act as a major source of bacterial
55 contamination, for instance, knives and cutting boards used with uncooked products such as raw meat
56 or poultry may contaminate cooked or ready-to-eat products, particularly if they are used without
57 being adequately cleaned and disinfected. Plastic and wooden made cutting boards are considered as
58 a major vehicle for bacterial cross contamination, particularly with deep cracks and scars on the
59 surface that may provide a suitable environment for bacteria to survive (Goh and others 2014).
60 Sanitizing with chemical agents is the most common and economical method to reduce bacterial count
61 to levels considered safe. Food contact sanitizers, which are used in food processing, handling,
62 preparation and service industry, are mainly used on surfaces that are normally come in contact with
63 food products and should only be applied to cleaned surfaces (Gaulin and others 2011; U.S.
64 Department of Agriculture 2013). However, in order to be authorized with disinfectant claims, food
65 contact sanitizers must reduce microbial contamination by 5-log₁₀ at 20 °C (U.S. Environmental
66 Protection Agency 1999; U.S. Food and Drug Administration 2009; Gaulin and others 2011; U.S.

67 Department of Agriculture 2013). Additionally, approved food sanitizers must be safe for use on food
68 contact surfaces, do not require a rinse after the sanitizing step (rated by the USDA as D2 sanitizers),
69 free of dyes and fragrances and EPA registered for sanitizing food contact surfaces (U.S.
70 Environmental Protection Agency 1999; U.S. Food and Drug Administration 2009; Gaulin and others
71 2011; U.S. Department of Agriculture 2013).

72 There are numerous commercial sanitizers that are approved to be used in food premises and
73 which may contain chlorine compounds, peroxide mixtures, quaternary ammonium compounds
74 (QUATS), acid anionic, hydrogen peroxide and iodine (Marriott 2006; U.S. Food and Drug
75 Administration 2009; Fraser and Pascall 2010; Gaulin and others 2011). Chlorine based sanitizers are
76 the most commonly used by the food service industry for many reasons; they are effective on a wide
77 variety of microorganisms, generally inexpensive, and considered the easiest sanitizers to prepare and
78 test with relatively stable efficacy (Fraser and Pascall 2010; Gaulin and others 2011; Monnin and
79 others 2012). However, chlorine based sanitizers are corrosive and may form toxic chlorine
80 byproducts if they applied at higher concentrations, and their bactericidal activity reduces in the
81 presence of organic matter (Fawell 2000; Fraser and Pascall 2010; Gaulin and others 2011; Monnin
82 and others 2012). QUATS based sanitizers are widely used as bactericidal agents in medical and food
83 environments in which they are generally applied at 200 mg/L (Pfundner 2011). They are colorless,
84 odorless, nontoxic, noncorrosive, nonirritating, stable at high temperature, active over a wide pH
85 range, and relatively remain effective in the presence of organic materials (Sundheim and others
86 1998; Fraser and Pascall 2010; Pfundner 2011). QUATS are slow-acting against some
87 microorganisms and they are more effective against Gram-positive bacteria with limited activity
88 against Gram-negative bacteria (Gaulin and others 2011). Organic acids are generally recognized as
89 safe (GRAS) antimicrobial agents, they could be used in a concentration of 1-3% without an effect

90 on food quality attributes (Raftari and others 2009). Organic acids, such as lactic acid, have been
91 approved by the Food Safety and Inspection Service of the United States Department of Agriculture
92 to be applied as antibacterial agents against different types of pathogenic bacteria and to inhibit
93 spoilage in food products (Skrivanova and others 2011; Lingham and others 2012).

94
95 Due to its antimicrobial properties, electrolyzed water (EW) has been applied to control
96 bacterial contamination on food products, non-food contact surfaces, and food processing surfaces,
97 including the equipment and utensils used in food preparation (Venkitanarayanan and others 1999;
98 Deza and others 2007; Hricova and others 2008; Fraser and Pascall 2010; Monnin and others 2012).
99 However, in addition to its advantages of reducing equipment corrosiveness and minimizing skin and
100 mucous irritation, using of neutral EW (NEW) that combines both acidic and basic EW may optimize
101 bactericidal effect due to the increase in penetration rate of NEW water through bacterial cell
102 membranes, its high oxidation reduction potential, availability of chlorine with the presence of OH⁻
103 as an active surfactant, and its longer storage life at neutral pH which reduces chlorine loss (Len and
104 others 2002; Monnin and others 2012).

105 The significance of this study was to investigate the bactericidal activity of neutral
106 electrolyzed water, quaternary ammonium, and lactic acid based solutions against *Salmonella*
107 Typhimurium, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Listeria monocytogenes* and
108 *Staphylococcus aureus* that were inoculated onto the surface of scarred polypropylene and wooden
109 food cutting boards. The study also examined the antimicrobial activity when using cutting boards in
110 preparation of raw chopped beef, chicken tenders or salmon fillet.

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113 **Materials and Methods**

114 **Bacterial strains and inoculum preparation**

115 All bacterial American Type Culture Collection (ATCC) strains were obtained from
116 Microbiologics, Inc. (St. Cloud, MN). *S. Typhimurium* ATCC 13311, *E. coli* O157:H7 ATCC 43888,
117 *L. monocytogenes* ATCC 19112 and *S. aureus* ATCC 29213 were individually cultured and activated
118 by inoculating each specific Kwik-Stik swab (Microbiologics, Inc.) into 50 mL of tryptic soy broth
119 TSB (Bacto™) and then were incubated at 37°C for 24 h. All strains were cultured to yield a cell
120 count of approximately 10⁹ CFU/mL. Bacterial suspensions were enumerated in duplicate using the
121 spread-plate technique in which a 1-mL aliquot of each dilution was divided into 5 aliquots of 0.2
122 mL and cultured on tryptic soy agar TSA (Bacto™). *C. jejuni* ATCC 29428 was activated by
123 inoculating the Kwik-Stik swab into 50 mL of Campylobacter enrichment broth consisting of
124 Campylobacter nutrient broth no. 2 (CM0067, Oxoid Ltd.) and supplemented with Campylobacter
125 growth supplement (SR0232E, Oxoid Ltd.). *C. jejuni* broth was then incubated in an anaerobic jar at
126 37 °C for 48 h under a microaerophilic atmosphere (~ 6 to 7% O₂) using CampyGen sachets (CN0025,
127 Oxoid Ltd.) (Al-Qadiri and others 2015). *C. jejuni* was cultured to obtain a cell count of
128 approximately 10⁹ CFU/mL, it was enumerated in duplicate using the spread-plate technique and
129 cultured on Campylobacter blood-free medium (modified CCDA Preston, CM0739, Oxoid Ltd.)
130 (Astorga and Alonso 2010; Al-Qadiri and others 2015).

131 After the appropriate incubation of bacterial cultures, 50 mL broth of each strain was
132 transferred under aseptic conditions to a sterile centrifuge tube and centrifuged for 15 min at 5000
133 rpm (3380 x g) to harvest bacterial cells (AccuSpin centrifuge, Thermo Fisher Scientific, Waltham,
134 MA). To eliminate any effect of broth components and bacterial metabolites, the resultant pellets
135 were resuspended in 50 mL of sterile saline solution (0.85 %; wt/vol NaCl). The tubes were then
136 centrifuged as before, and the resulting pellets of the five strains were then resuspended in 50-mL

137 aliquots and centrifuged for a second time as described above. The supernatant was decanted and the
138 resulting washed pellets were resuspended in sterile 10-mL aliquots, which were then used to
139 inoculate the surfaces of the cutting boards. To prepare the approximate cell suspension inocula of
140 each strain, culturing schemes for bacterial cells were based upon separate experiments in which the
141 approximate cell inocula for the five bacterial strains were preliminary determined.

142 **Cutting boards and inoculation process**

143 Polypropylene and maple-hardwood cutting boards were used in this study to examine the
144 antibacterial activity profiles. To simulate normal usage, the entire one surface of each cutting board
145 was scarred by metal grater then by kitchen pizza-cutter (50 times in cross sectional directions), the
146 surface was then sharply marked into squares (10 x 10 cm² each). The boards were thoroughly rinsed
147 with sterile deionized water and sprayed with 70% ethyl alcohol. The polypropylene cutting boards
148 were wrapped in aluminum foil and sterilized by autoclaving at 121 °C for 20 min. The wooden
149 cutting boards were placed in boiling water for 30 min and then aseptically wrapped using sterile
150 aluminum foil sheets. Swabbing of these cutting boards showed no bacterial recovery prior to each
151 experimental trial.

152 On each cutting board surface, a volume of 5 mL of the previously prepared bacterial culture
153 (approximately 10⁹ CFU/mL) was applied separately at ambient temperature (22 °C) and evenly
154 spread over the entire surface using a sterile wet cotton swab. Following inoculation process, the
155 cutting boards were dried under aseptic conditions in a laminar flow hood for 30 min at ambient
156 temperature. Another group of the cutting boards was used to separately prepare raw chopped beef,
157 chicken tenders or salmon fillet. Prepackaged raw beef, chicken and salmon portions were purchased
158 from a local retail store and kept at 4 °C overnight. No preparation of washing, removal of fat or skin
159 tissue was undertaken before processing. Two pounds of meat samples were prepared using a kitchen

160 knife, meat pieces were rubbed several times over the surface of each cutting board, processing time
161 was within 10-15 min. Meat pieces were then removed and the cutting boards with meat juices were
162 air dried under a laminar flow hood for 1 h at ambient temperature to simulate the normal use in food
163 service establishments.

164 The initial viable bacterial load on the inoculated air-dried surfaces was determined by
165 swabbing two squares (the corner plus the middle, 100 cm² each) using individual sterile wet cotton
166 swabs that were previously prepared in a 5 mL sterile D/E Neutralizing Broth (Difco™). The swabs
167 were washed and 1 mL of appropriate dilutions was enumerated in duplicate using the spread-plate
168 technique. A 1-mL aliquot was divided into 5 aliquots of 0.2 mL and cultured on tryptic soy agar
169 TSA (Bacto™) or Campylobacter blood-free medium as described above. The mean viable bacterial
170 counts were determined as log CFU/100 cm². For cutting boards that had been used to prepare meat
171 pieces, the initial viable count was determined as above, however, swabbing was performed after
172 rinsing the surfaces with sterile deionized water to remove any organic residues followed by air drying
173 for 30 min as described below. TSA (Bacto™) was used to determine total viable counts (log
174 CFU/100 cm²), plates were incubated at 37 °C and examined for 72 h.

175 **Preparation of treatment solutions**

176 Three antimicrobial treatments were prepared: neutral electrolyzed water (NEW), quaternary
177 ammonium (QUAT) and lactic acid based solutions. A commercial NEW (Aquaox Disinfectant 275)
178 was provided from Aquaox Industries Inc. Fontana, CA 92336. The active ingredient of the Aquaox
179 NEW stock solution is hypochlorous acid (0.0275%) that is generated electrochemically by
180 electrolysis of a dilute sodium chloride solution passing through an electrolytic cell at neutral pH. For
181 the treatment of cutting boards, Aquaox NEW was diluted in sterile deionized water to obtain a final
182 free available chlorine (FAC) content of 200 mg/L, a pH of 6.6 and an oxidation-reduction potential

183 (ORP) of 805 mV. Treatment solution was kept refrigerated and used within 3 h of preparation. The
184 pH, ORP and FAC content were measured by a pH meter (FE20, Mettler-Toledo, Columbus, OH,
185 USA), a pocket sized redox meter (HI 98201, HANNA[®] Instruments, Ann Arbor, Michigan, USA)
186 and a digital colorimeter (Colorimeter[™] Analysis System, Hach Co., Loveland, CO, USA),
187 respectively, according to the manufacturer instructions. A commercial EPA registered D2 classified
188 QUAT based antimicrobial solution was used as a second treatment option. The active ingredients of
189 the QUAT stock solution are 5% as alkyl (60% C₁₄, 30% C₁₆, 5% C₁₂, 5% C₁₈) dimethyl benzyl
190 ammonium chlorides and 5% as alkyl (68% C₁₂, 32% C₁₄) ethylbenzyl ammonium chlorides. For the
191 treatment of cutting boards, QUAT stock solution was diluted in sterile deionized water according to
192 manufacturer instructions to obtain a final active QUAT solution of 200 mg/L. As a third treatment
193 solution, a commercial household lactic acid based antibacterial detergent was used in which L-Lactic
194 acid (2%) is the main active antibacterial ingredient (98% as inert ingredients). The treatment solution
195 was prepared according to the manufacturer instructions by diluting 100 mL detergent in 2 L sterile
196 deionized water.

197 **Sanitization treatment and microbial recovery**

198 For cutting boards that were inoculated with bacterial strains, each air-dried surface was
199 entirely sprayed three times within 30 seconds (from top to bottom and from right to left) with 30 mL
200 of the previously prepared NEW, QUAT, or lactic acid based solutions at ambient temperature. For
201 cutting boards that had been used to prepare meat pieces, they were aseptically rinsed with a sterile 1
202 L deionised water (using a sterile stainless tray) to remove any organic residues followed by air drying
203 under aseptic conditions in a laminar flow hood for 30 min at ambient temperature. After drying, they
204 were sprayed with 30 mL treatment solutions as described above.

205 To recover surviving bacteria, viable bacterial counts were determined within 1, 3 and 5 min
206 as treatment time intervals. Two squares (100 cm² each) were individually selected for each time
207 interval and swabbed by using separate sterile wet cotton swabs that were previously prepared in a 5
208 mL sterile D/E Neutralizing Broth (Difco™). Each square was swabbed in three directions, vertical,
209 horizontal and diagonal. The swabs were then washed and 1 mL of the homogenized suspension was
210 serially diluted (dilution range: 10⁰ to 10⁻⁴) in 9 mL of sterile 0.1% peptone water (Bacto™). Samples
211 were examined in duplicate using the spread-plate technique. A 1-mL aliquot of each dilution was
212 divided into 5 aliquots of 0.2 mL, which then they were evenly spread on TSA (Bacto™) for
213 enumeration of *S. Typhimurium*, *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* (Al-Qadiri and
214 others 2006, 2008). Plates were then incubated at 37°C for 24-48 h. Campylobacter blood-free
215 medium (modified CCDA Preston, CM0739, Oxoid Ltd.) was used to enumerate surviving *C. jejuni*
216 (Astorga and Alonso, 2010). Plates were incubated at 37 °C for 48 h under microaerophilic
217 conditions. The mean viable bacterial counts were determined as log CFU/100 cm². TSA (Bacto™)
218 was used to recover surviving microorganisms on treated surfaces used to prepare meat pieces in
219 which microbial swabbing was performed as above (dilution range: 10⁰ to 10⁻²). Plates were incubated
220 at 37°C and examined for 72 h. The mean viable counts were determined as log CFU/100 cm².
221 Surfaces that were not treated with antimicrobial solutions served as controls (baseline reading). All
222 tests were carried out in triplicate.

223 **Statistical analysis**

224 The experiment consisted of three independent replicate trials ($n = 3$) and each reported value
225 is the mean viable count \pm standard error (SE) of the results of three replicate treatments per
226 experimental trial. An analysis of variance, using the mixed-effects procedure for bacterial counts,
227 was conducted with SAS software (SAS Institute, 2011). Polypropylene or wood, treatment solution,

228 and treatment time were treated as fixed effects. Subjects were random samples from the target
229 population and, therefore, were treated as random effects in the model. The interaction among fixed-
230 effect variables was analyzed. The Kenward and Roger method was used to evaluate the denominator
231 degrees of freedom (Kenwardroger = DDFM). In order to adjust the estimated standard deviations
232 for fixed effects and interaction effects (Littell et al., 2006), the level of significance was set at a *P*
233 value of < 0.05. Post hoc multiple pairwise comparisons of treatment group means were performed
234 with the Tukey-Kramer adjustment (Tukey's honestly significant difference test) to control the type
235 I error rate.

236 **Results and Discussion**

237 Microbial contamination of cutting boards used in food preparation may pose a health threat
238 of causing foodborne illnesses when sanitizing procedures are not applied efficiently. Different
239 chemical sanitizers are used to reduce microbial loads on cutting boards surfaces; however, several
240 factors may restrict their applications. Limitations may include effective concentration to be applied,
241 contact time, active ingredients, residual effect, formation of toxic byproducts, type of
242 microorganisms present and nature of organic and inorganic residues on the surface (Fraser and
243 Pascall 2010). For the current study, it might be the first in investigation with comparison the
244 antimicrobial activity of NEW, QUAT and lactic acid based formulations against microbial
245 contamination of laboratory inoculated scarred polypropylene and wooden cutting boards surfaces at
246 ambient temperature. As shown in Tables 1 and 2, NEW and QUAT treatments showed a broad
247 spectrum of action over the studied bacterial strains. There were significant differences ($P < 0.05$) in
248 bacterial reductions with regard to contact time and which maximized after 5 min of treatment. Within
249 the first minute of treatment, NEW and QUAT solutions caused more than 3 log/100 cm² bacterial
250 reductions on polypropylene surfaces whereas less than 3 log reductions were achieved on wooden

251 surfaces. After 5 min of treatment, more than 5 log reductions were achieved in all bacterial strains
252 inoculated onto polypropylene surfaces, however, Gram (+) bacteria were more sensitive to both
253 antimicrobial solutions. Using NEW and QUAT solutions within 5 min reduced Gram-negative
254 bacteria by 4.58-4.85 log compared to more than 5 log reductions in Gram-positive bacteria
255 inoculated onto wooden surfaces. Obviously, there was no significant difference ($P > 0.05$) between
256 NEW and QUAT treatments on both cutting board surfaces; however, treatment effectiveness against
257 inoculated bacteria was higher on polypropylene compared to wooden surfaces.

258 Our findings are consistent with a previous study reported that rinsing of contaminated cutting
259 boards in either NEW or sodium hypochlorite NaClO solutions (~64 mg/L) revealed no significant
260 differences between the final populations of each bacterial strain with regard to the treatment
261 solutions, however, a significant difference was found between the decontamination of plastic and
262 wooden surfaces. In plastic boards, the initial bacterial populations decreased by approximately 5.4
263 log CFU/50 cm² after 1 min, however, in wooden boards the initial bacterial populations reduced by
264 2.5 log, and when the rinsing time was increased to 5 min, populations were reduced by about 4 log
265 (Deza and others 2007). In a study performed to investigate bacterial retention and cleanability of
266 plastic and wooden cutting boards, it was revealed that wooden boards could absorb bacterial
267 suspension in which the inner part of the wood might still remain wet and retain most of the bacteria
268 although the surface appeared dry (Welkers and others 1997).

269 As sanitizers, QUATS are commonly applied at 200 mg/L to food contact surfaces, the
270 solution is allowed to dry in which a residual effect may remain and provide antimicrobial activity
271 until degradation occurs (Pfundner 2011). The germicidal activity of QUATS is mainly due to the
272 binding of the positively charged cations with the acidic phospholipids in the microbial cell
273 membrane to block the transportation of nutrients and discharge of waste into and out of the

274 cytoplasm (Block 2001; McBain and others 2004). In this study, we found that QUAT solution was
275 more effective against *L. monocytogenes* and *S. aureus* compared to Gram-negative bacteria.
276 Although QUAT formulations are effective against a wide range of microbes, it was revealed that
277 their antimicrobial action is more effective against Gram-positive bacteria (Block 2001; McBain and
278 others 2004; Pfunter 2011). It was reported that QUATS are more effective against *L.*
279 *monocytogenes* with limited effectiveness against most Gram-negative bacteria except *Salmonella*
280 spp. and *E. coli* (Holah and others 2002; Gaulin and others 2011).

281 Lactic acid treatment was significantly less effective ($P < 0.5$) compared to NEW and QUAT
282 treatments. As shown in Tables 1 and 2, less than 2 log reductions were achieved within the first
283 minute of treatment. As found above, lactic acid was more effective against inoculated bacteria on
284 polypropylene surfaces, the highest log reductions achieved after 5 min were 2.2-2.75 and 1.9-2.45
285 log/100 cm² for polypropylene and wooden surfaces, respectively, and in which bacterial inhibition
286 was more effective against Gram-positive bacteria. L-(+)-Lactic acid is known as 2-
287 hydroxypropanoic acid, which is a GRAS organic acid belonging to carboxylic acids family (U.S.
288 Environmental Protection Agency 2009). Lactic acid has a relatively limited antimicrobial efficacy;
289 the undissociated form passively diffuses into the cytoplasm causes internal pH to decrease, protein
290 denaturation, and disruption of proton motive force (Cherrington and others 1990; Culver and others
291 2014). The relative sensitivity of Gram-positive bacteria to lactic acid could be linked to the structure
292 of the cell wall which does not possess an outer membrane, which as a result may decrease intrinsic
293 resistance against organic acids (Raftari and others 2009). Lactic acid solutions are widely used as
294 general decontaminants to control microbial contamination in meat carcass and minimally processed
295 produce (Barboza and others 2002; Raftari and others 2009; Sagong and others 2011). In a previous
296 study, 0.5% lactic acid was used for 2 min to reduce microbial contamination in iceberg lettuce in

297 which *E. coli* and *L. monocytogenes* were reduced by 2.7 and 2.0 log CFU/g, respectively. However,
298 treatment at 1% for 5 min only raised decontamination efficacy to 3.0 and 2.2 log CFU/g, respectively
299 (Akbas and Olmez 2007). Sasong and others (2011) reported that washing of iceberg lettuce with 1%
300 lactic acid for 5 min decreased bacterial counts of *E. coli* O157:H7, *Salmonella* Typhimurium, and
301 *L. monocytogenes* by 1.45, 1.39, and 1.17 log CFU/g, respectively.

302 To simulate normal application, the three antimicrobial solutions were used at the same
303 concentrations and time intervals to reduce microbial counts on cutting board surfaces that had been
304 used to prepare raw chopped beef, chicken tenders, and salmon fillet. As shown in Tables 3 and 4,
305 there were no significant differences ($P > 0.05$) in microbial reductions between NEW and QUAT
306 treatments. Within the first minute of treatment, less than 2 log/100 cm² of microbial reductions were
307 achieved in both board surface materials, however, the antimicrobial effectiveness was more intense
308 against microbial loads on polypropylene compared to wooden surfaces. After 3 min of treatment,
309 microbial loads were further reduced to less than 1 log and were not detected due to lethal injury after
310 5 min of treatment in both surface materials. Lactic acid treatment was less effective with limited
311 efficacy, about 0.5, 1, and < 2 log/100 cm² of microbial reductions were achieved within 1, 3 and 5
312 min of treatment, respectively, in which effectiveness was higher in polypropylene boards.
313 Obviously, the antimicrobial effectiveness of the three treatments in both cutting board types was
314 significantly restricted when solutions applied on surfaces used to prepare raw meat compared to
315 bacterial inoculated surfaces. In a study used inoculated food intermediate to contaminate scarred
316 hardwood cutting boards, it was found that manual washing and rinsing followed by sanitization with
317 NEW and NaClO (~100 mg/L) produced similar levels of bacterial inactivation in which the
318 population reductions were less than 5 log CFU/100 cm² (3.4 and 3.6 log for *E. coli*, and 4.1 and 3.9
319 log for *L. innocua*, respectively) (Monnin and others (2012). Organic matter may inactivate and

320 reduce the effectiveness of chemical sanitizers; accordingly, to achieve the 5-log reduction (99.999%)
321 in microbial loads, chemical sanitizers must be applied to surfaces that are free of organic matter
322 (Gaulin and others 2011; Pfunter and others 2011). It was reported that organic matter from food
323 residues (grease and proteins) may harbor bacteria and prevent sanitizers to be in direct physical
324 contact with surfaces to be sanitized (Fraser and Pascall, 2010).

325 **Conclusions**

326 This study revealed that NEW could be used as an effective antimicrobial treatment
327 alternative to commonly used chemical sanitizers such as QUATS. NEW also showed a broad
328 spectrum of action against the evaluated bacterial strains inoculated onto both types of surface
329 materials. However, the obtained results demonstrated that the examined treatments were more
330 effective against microbial contamination on polypropylene compared to wooden surfaces. It was
331 found that using of lactic acid as a sanitizer may not be a suitable option to decontaminate food cutting
332 boards. A considerable decline in antimicrobial effectiveness was observed when both cutting board
333 types had been used to prepared raw meat, which could be due to the presence of food organic
334 residues.

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Table 1-Mean viable bacterial counts (\log_{10} CFU/100 cm²) recovered on polypropylene cutting boards surfaces after treatment with NEW, QUAT and lactic acid based antimicrobial solutions^{1, 2}.

Treatment solution- Time (min)	Bacterial strain				
	<i>S. Typhimurium</i>	<i>E. coli</i> O157:H7	<i>C. jejuni</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>
<u>NEW</u>					
0	8.15 ± 0.07 ^a	8.20 ± 0.07 ^a	7.50 ± 0.06 ^a	8.23 ± 0.07 ^a	8.30 ± 0.05 ^a
1	4.43 ± 0.05 ^e (3.72)	4.68 ± 0.05 ^d (3.52)	4.23 ± 0.04 ^d (3.27)	4.38 ± 0.04 ^e (3.85)	4.55 ± 0.05 ^d (3.75)
3	3.65 ± 0.05 ^g (4.50)	3.95 ± 0.04 ^e (4.25)	3.18 ± 0.07 ^f (4.32)	3.35 ± 0.05 ^f (4.88)	3.43 ± 0.05 ^e (4.87)
5	2.83 ± 0.04 ⁱ (5.32)	3.01 ± 0.04 ^f (5.19)	2.35 ± 0.04 ^g (5.15)	2.58 ± 0.04 ^g (5.65)	2.75 ± 0.05 ^f (5.55)
<u>QUAT</u>					
0	8.10 ± 0.06 ^a	8.13 ± 0.07 ^a	7.43 ± 0.06 ^a	8.33 ± 0.07 ^a	8.15 ± 0.05 ^a
1	4.73 ± 0.06 ^d (3.37)	4.73 ± 0.05 ^d (3.40)	4.33 ± 0.04 ^d (3.10)	4.70 ± 0.04 ^d (3.63)	4.38 ± 0.05 ^d (3.77)
3	3.95 ± 0.05 ^f (4.15)	4.00 ± 0.04 ^e (4.13)	3.40 ± 0.05 ^e (4.03)	3.50 ± 0.05 ^f (4.83)	3.45 ± 0.05 ^e (4.70)
5	3.05 ± 0.04 ^h (5.05)	3.02 ± 0.05 ^f (5.11)	2.41 ± 0.04 ^g (5.02)	2.73 ± 0.04 ^g (5.60)	2.78 ± 0.05 ^f (5.37)
<u>Lactic acid</u>					
0	8.05 ± 0.07 ^a	8.25 ± 0.07 ^a	7.38 ± 0.06 ^a	8.20 ± 0.07 ^a	8.25 ± 0.05 ^a
1	6.58 ± 0.06 ^b (1.47)	6.65 ± 0.05 ^b (1.60)	5.85 ± 0.04 ^b (1.53)	6.33 ± 0.04 ^b (1.87)	6.34 ± 0.04 ^b (1.91)
3	5.95 ± 0.05 ^c (2.10)	6.20 ± 0.04 ^c (2.05)	5.35 ± 0.05 ^c (2.03)	5.63 ± 0.05 ^c (2.57)	5.80 ± 0.05 ^c (2.45)
5	5.76 ± 0.04 ^c (2.29)	6.02 ± 0.04 ^c (2.23)	5.18 ± 0.04 ^c (2.20)	5.45 ± 0.04 ^c (2.75)	5.63 ± 0.04 ^c (2.62)

¹ Values are the means of three independent replicate trials ± standard error, with \log_{10} reductions (CFU/100 cm²) presented in parentheses.

² Within the same column, treatment means without shared superscripts are significantly different (Tukey's HSD test, $P < 0.05$).

Table 2-Mean viable bacterial counts (\log_{10} CFU/100 cm^2) recovered on wooden cutting boards surfaces after treatment with NEW, QUAT and lactic acid based antimicrobial solutions^{1, 2}.

Treatment solution- Time (min)	Bacterial strain				
	<i>S. Typhimurium</i>	<i>E. coli</i> O157:H7	<i>C. jejuni</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>
<u>NEW</u>					
0	7.90 ± 0.07 ^a	7.98 ± 0.07 ^a	7.10 ± 0.05 ^a	8.00 ± 0.07 ^a	7.98 ± 0.05 ^a
1	5.15 ± 0.06 ^d (2.75)	5.30 ± 0.05 ^d (2.68)	4.30 ± 0.04 ^d (2.80)	5.05 ± 0.05 ^d (2.95)	5.17 ± 0.05 ^d (2.81)
3	4.05 ± 0.05 ^e (3.85)	4.10 ± 0.04 ^e (3.88)	3.21 ± 0.05 ^e (3.89)	3.90 ± 0.05 ^e (4.10)	3.82 ± 0.05 ^f (4.16)
5	3.10 ± 0.04 ^f (4.80)	3.13 ± 0.04 ^f (4.85)	2.34 ± 0.04 ^f (4.76)	2.85 ± 0.04 ^f (5.15)	2.87 ± 0.05 ^g (5.11)
<u>QUAT</u>					
0	7.87 ± 0.07 ^a	7.95 ± 0.07 ^a	7.00 ± 0.06 ^a	7.98 ± 0.07 ^a	8.05 ± 0.06 ^a
1	5.18 ± 0.06 ^d (2.69)	5.15 ± 0.05 ^d (2.80)	4.23 ± 0.04 ^d (2.77)	5.11 ± 0.04 ^d (2.87)	4.94 ± 0.05 ^e (3.11)
3	4.10 ± 0.05 ^e (3.77)	4.08 ± 0.04 ^e (3.87)	3.28 ± 0.05 ^e (3.72)	3.95 ± 0.05 ^e (4.03)	3.78 ± 0.04 ^f (4.27)
5	3.25 ± 0.04 ^f (4.62)	3.25 ± 0.04 ^f (4.70)	2.42 ± 0.04 ^f (4.58)	2.93 ± 0.04 ^f (5.05)	2.90 ± 0.05 ^g (5.15)
<u>Lactic acid</u>					
0	7.85 ± 0.07 ^a	7.90 ± 0.07 ^a	7.03 ± 0.06 ^a	7.90 ± 0.06 ^a	7.93 ± 0.07 ^a
1	6.65 ± 0.06 ^b (1.20)	6.65 ± 0.05 ^b (1.25)	5.73 ± 0.04 ^b (1.30)	6.33 ± 0.04 ^b (1.57)	6.28 ± 0.05 ^b (1.65)
3	6.03 ± 0.05 ^c (1.82)	6.15 ± 0.04 ^c (1.75)	5.25 ± 0.05 ^c (1.78)	5.70 ± 0.05 ^c (2.20)	5.65 ± 0.06 ^c (2.28)
5	5.85 ± 0.04 ^c (2.00)	5.99 ± 0.04 ^c (1.91)	5.07 ± 0.04 ^c (1.96)	5.52 ± 0.04 ^c (2.38)	5.48 ± 0.04 ^c (2.45)

¹ Values are the means of three independent replicate trials ± standard error, with \log_{10} reductions (CFU/100 cm^2) presented in parentheses.

² Within the same column, treatment means without shared superscripts are significantly different (Tukey's HSD test, $P < 0.05$).

Table 3-Mean viable counts (\log_{10} CFU/100 cm^2) recovered on polypropylene cutting boards surfaces used to prepare meat samples after treatment with NEW, QUAT and lactic acid based antimicrobial solutions^{1, 2, 3}.

Treatment solution- Time (min)	Meat sample		
	Chopped beef	Chicken tenders	Salmon fillet
<u>NEW</u>			
0	3.10 ± 0.05 ^a	3.28 ± 0.05 ^a	3.80 ± 0.05 ^a
1	1.43 ± 0.04 ^d (1.67)	1.67 ± 0.05 ^d (1.61)	2.05 ± 0.04 ^d (1.75)
3	<1 ^e	<1 ^e	<1 ^e
5	ND	ND	ND
<u>QUAT</u>			
0	2.97 ± 0.05 ^a	3.15 ± 0.05 ^a	3.70 ± 0.05 ^a
1	1.37 ± 0.04 ^d (1.60)	1.62 ± 0.05 ^d (1.53)	2.00 ± 0.04 ^d (1.70)
3	<1 ^e	<1 ^e	<1 ^e
5	ND	ND	ND
<u>Lactic acid</u>			
0	3.01 ± 0.05 ^a	3.20 ± 0.05 ^a	3.65 ± 0.05 ^a
1	2.34 ± 0.04 ^b (0.67)	2.60 ± 0.06 ^b (0.60)	3.03 ± 0.04 ^b (0.62)
3	1.81 ± 0.04 ^c (1.20)	2.10 ± 0.05 ^c (1.10)	2.56 ± 0.03 ^c (1.09)
5	1.28 ± 0.03 ^d (1.73)	1.50 ± 0.03 ^d (1.70)	1.93 ± 0.03 ^d (1.72)

¹ Values are the means of three independent replicate trials ± standard error, with \log_{10} reductions (CFU/100 cm^2) presented in parentheses.

² Within the same column, treatment means without shared superscripts are significantly different (Tukey's HSD test, $P < 0.05$).

³ ND, not detected due to lethal injury.

Table 4-Mean viable counts (\log_{10} CFU/100 cm^2) recovered on wooden cutting boards surfaces used to prepare meat samples after treatment with NEW, QUAT and lactic acid based antimicrobial solutions^{1, 2, 3}.

Treatment solution- Time (min)	Meat sample		
	Chopped beef	Chicken tenders	Salmon fillet
<u>NEW</u>			
0	3.17 ± 0.05 ^a	3.45 ± 0.05 ^a	3.87 ± 0.05 ^a
1	1.75 ± 0.05 ^d (1.42)	2.19 ± 0.05 ^d (1.26)	2.46 ± 0.04 ^d (1.41)
3	<1 ^e	<1 ^e	<1 ^e
5	ND	ND	ND
<u>QUAT</u>			
0	3.21 ± 0.05 ^a	3.40 ± 0.05 ^a	3.78 ± 0.05 ^a
1	1.83 ± 0.04 ^d (1.38)	2.23 ± 0.04 ^d (1.17)	2.33 ± 0.04 ^d (1.45)
3	<1 ^e	<1 ^e	<1 ^e
5	ND	ND	ND
<u>Lactic acid</u>			
0	3.15 ± 0.05 ^a	3.35 ± 0.05 ^a	3.75 ± 0.05 ^a
1	2.65 ± 0.04 ^b (0.50)	2.87 ± 0.06 ^b (0.48)	3.18 ± 0.04 ^b (0.57)
3	2.15 ± 0.04 ^c (1.00)	2.50 ± 0.05 ^c (0.85)	2.83 ± 0.03 ^c (0.92)
5	1.65 ± 0.03 ^d (1.50)	2.05 ± 0.03 ^d (1.30)	2.28 ± 0.03 ^d (1.47)

¹ Values are the means of three independent replicate trials ± standard error, with \log_{10} reductions (CFU/100 cm^2) presented in parentheses.

² Within the same column, treatment means without shared superscripts are significantly different (Tukey's HSD test, $P < 0.05$).

³ ND, not detected due to lethal injury.