

1 **Running title:** *Salmonella* reduction in frozen NRTE breaded chicken products

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4 **Antimicrobials for Reduction of *Salmonella* Contamination in Uncooked, Surface-Browned**
5 **Breaded Chicken Products**

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17 **Key words:** *Salmonella*, antimicrobials, uncooked surface-browned breaded chicken products

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ABSTRACT

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Surface-browned but uncooked, frozen breaded chicken products have been associated with salmonellosis outbreaks due to inadequate or no cooking of the products before consumption. This study evaluated the effect of three antimicrobials against *Salmonella* during manufacture of a surface-browned, uncooked, frozen breaded chicken meat product. Fresh chicken breast meat portions (5 × 5 × 5 cm) were inoculated (4-5 log CFU/g) with *Salmonella* and mixed with caprylic acid (CAA; 0.5 and 1.0%), carvacrol (CAR; 0.3 and 0.5%), ε-polylysine (POL; 0.125 and 0.25%) or distilled water (control). Sodium chloride (1.2%) and sodium tripolyphosphate (0.3%) were added to all treatments followed by grinding of the mixtures (5% total moisture enhancement level) and forming into 9 × 5 × 3 cm portions. The products were breaded and surface-browned by oven baking (208°C, 15 min) or deep frying in vegetable oil (190°C, 15 s), packaged in polyethylene bags, and stored at -20°C (7 days). Total reductions of inoculated *Salmonella* in untreated control oven- or fryer-browned products after frozen storage were 1.2 and 0.8 log CFU/g, respectively. In comparison, treatment with CAA, CAR or POL reduced initial pathogen counts by 3.3 to >4.5, 4.1 to >4.7, and 1.1 to 1.6 log CFU/g, respectively, irrespective of antimicrobial concentration and browning method. Treatment with 1.0% CAA (oven-browned) or 0.5% CAR (oven/fryer-browned) reduced *Salmonella* to non-detectable levels (<0.3 log CFU/g) in stored frozen products. These data may be useful in the development of suitable antimicrobial treatments to reduce the risk of *Salmonella* contamination in surface-browned, uncooked, frozen breaded chicken products.

41 Frozen, breaded chicken products containing raw poultry that appear ready-to-eat but in fact
42 are only surface-browned, include raw, frozen chicken nuggets, strips, and stuffed entrees (e.g.,
43 chicken cordon bleu, chicken Kiev) (22). Such not-ready-to-eat (NRTE) chicken products have
44 been linked to salmonellosis outbreaks in the United States (21), Canada (8, 17), and Australia
45 (14). Manufacture of such products involves use of raw chicken meat that undergoes particle size
46 reduction to improve protein extraction and binding of meat pieces with the addition of binding
47 ingredients, such as salt and phosphates. Once the product is formed, it undergoes a partial
48 cooking/browning (fried or baked) step to maintain the shape of the product and induce a
49 desirable golden-brown color prior to freezing and packaging; however, the browning step is not
50 a complete lethality step and is not intended to fully cook the product (3, 19).

51 Since the chicken meat used during manufacture of breaded chicken products is raw, the
52 bacteriological quality of these products should be considered the same as raw poultry (2, 10).
53 Typical control strategies for *Salmonella* in raw chicken products involve chemical antimicrobial
54 interventions applied as rinses, primarily at the slaughter facility (1, 16). However, this process
55 does not eliminate *Salmonella* because raw chicken meat can become cross-contaminated or
56 recontaminated during further processing steps (3). Thus, the raw chicken meat used to
57 manufacture these processed chicken products has a reasonable likelihood of being contaminated
58 with *Salmonella* after which there is no other lethality intervention prior to consumer cooking. A
59 study by Bucher et al. (3) found 27% (n=92) of retail and wholesale raw, frozen chicken nugget
60 and chicken strip samples positive for *Salmonella*.

61 The fact that these products do not appear raw, and sometimes are placed in close proximity
62 to ready-to-eat (i.e., fully cooked) processed chicken products in retail display cases (20), may
63 lead consumers to treat them with less precaution than they typically would a visibly raw

64 product. Therefore, there is still concern that consumers may undercook these products, making
65 them a significant risk factor in contracting foodborne salmonellosis. Hence, there is a need for
66 the industry to take additional measures to reduce the risk of *Salmonella* contamination in these
67 types of products. Despite the risk of foodborne illness arising from consumption of
68 undercooked, raw, frozen processed chicken products, there has been very little work
69 investigating interventions that can be applied to these types of products to reduce the risk of
70 *Salmonella*. Therefore, the objective of this study was to evaluate the antimicrobial effects
71 against *Salmonella* of caprylic acid, carvacrol, and ϵ -polylysine, applied individually, on raw
72 chicken meat intended for manufacture of a frozen, surface-browned, uncooked, breaded chicken
73 product.

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MATERIALS AND METHODS

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77 **Bacterial strains and inoculum preparation.** The inoculum was comprised of seven
78 *Salmonella* isolates of chicken or turkey origin (kindly provided by Dr. Vijay Juneja, Microbial
79 Food Safety Research Unit, ERRC-ARS-USDA, Wyndmoor, PA), and included *Salmonella*
80 Hadar FSIS 064/VJS6 (chicken), *Salmonella* Hadar FSIS MF61777/VJS19 (turkey), *Salmonella*
81 Kentucky FSIS 044/VJS2 (chicken), *Salmonella* Kentucky FSIS 062/VJS1 (chicken), *Salmonella*
82 Muenster FSIS MF61976/VJS15 (turkey), *Salmonella* Reading FSIS MF58210/VJS17 (turkey),
83 and *Salmonella* Thompson FSIS 132/VJS7 (chicken). These *Salmonella* serotype strains formed
84 colonies with black centers on xylose lysine deoxycholate (XLD) agar (Acumedia, Lansing, MI)
85 indicating hydrogen sulfide production. The strains were individually cultured and subcultured in
86 10 ml tryptic soy broth (Difco, Becton Dickinson, Sparks, MD) for 18-24 h at 35°C. The cell

87 cultures were then combined, harvested by centrifugation (4,629×g, 15 min, 4°C; Eppendorf
88 model 5810 R, Brinkmann Instruments Inc., Westbury, NY) and washed twice in 10 ml
89 phosphate-buffered saline (PBS, pH 7.4; 0.2 g/liter KH₂PO₄, 1.5 g/liter Na₂HPO₄·7H₂O,
90 8.0 g/liter NaCl, and 0.2 g/liter KCl). The washed cell pellet was resuspended in 70 ml PBS and
91 further diluted, in PBS, to a concentration of 6-7 log CFU/ml.

92

93 **Inoculation, treatment, product preparation, and storage.** Fresh, boneless, skinless
94 chicken breasts were purchased directly from a poultry processing facility in Colorado. If not
95 used within 24 h, the chicken breasts were vacuum-packaged and stored at -20°C. When needed,
96 they were thawed at 4°C for approximately 48 h before use. The chicken breast meat was cut into
97 pieces (approximately 5 × 5 × 5 cm), and batches of 2 kg were inoculated with 20 ml of the
98 *Salmonella* inoculum to a target level of 4-5 log CFU/g. The chicken meat and inoculum were
99 thoroughly mixed for 2 min using a KitchenAid Professional 600™ mixer (St. Joseph, MI) at a
100 speed setting of “stir”, and then left to stand at 4°C for 30 min for bacterial cell attachment. The
101 inoculated batches (2 kg) of chicken meat were then treated with 20 ml of one of the following
102 treatments; as indicated, two concentrations of each antimicrobial were tested: (i) sterile distilled
103 water (control), (ii) caprylic acid (CAA, 0.5 and 1.0% v/w; Fisher Scientific, Hampton, NH), (iii)
104 carvacrol (CAR, 0.3 and 0.5% v/w; Acros Organics, Geel, Belgium), and (iv) ε-polylysine (POL,
105 0.125 and 0.25% v/w; Chisso Corporation, Minamata, Japan). These antimicrobials were
106 selected for evaluation based on results of a screening study (unpublished data) in which four
107 concentration levels each of 10 antimicrobials (allyl isothiocyanate, caprylic acid, carvacrol,
108 citric acid, grapefruit distilled terpene, malic acid, oregano oil, ε-polylysine, sodium citrate, and
109 sodium lactate) were evaluated for antimicrobial effects against *Salmonella* inoculated on raw

110 chicken portions. Based on the results of the screening study, caprylic acid and carvacrol were
111 found to be the most effective acid and essential oil, respectively (unpublished data). ϵ -
112 Polylysine, a cationic surfactant, was not as effective against the pathogen as caprylic acid or
113 carvacrol, but it was included in the present study based on previous published reports (6, 11, 13)
114 of its antimicrobial activity against *Salmonella* and other foodborne pathogens.

115 The inoculated chicken portions, in the present study, were mixed with the distilled water or
116 antimicrobial solution for 5 min using the KitchenAid mixer, followed by addition and mixing (5
117 min) of sodium chloride (Fisher Scientific) and sodium tripolyphosphate (kindly provided by BK
118 Giulini Corporation, Simi Valley, CA) to yield concentrations of 1.2 and 0.3% (w/w),
119 respectively, in the final product. The mixture, with a total moisture enhancement level of 5%,
120 was then ground (0.6 cm grinder plate) with an electric meat grinder (TSM#8, The Sausage
121 Maker Inc., Buffalo, NY), and formed into rectangular (9 cm length \times 5 cm width \times 3 cm height)
122 150 g portions. These product dimensions were representative of commercially-available frozen,
123 NRTE breaded chicken products found in local supermarkets. The portions were then brushed
124 with beaten pasteurized egg whites (All Whites, Crystal Farm, Lake Mills, WI) and rolled in
125 plain (i.e., unseasoned) breadcrumbs (Kroger, Cincinnati, OH), followed by browning for 15 min
126 (900 s) in a standard kitchen oven (Magic Chef, Maytag Corp., Newton, IA) set at 208°C. The
127 temperature of the oven chamber and the geometric center of products was monitored and
128 recorded at 1 s intervals during browning, using type-K thermocouples and PicoLog data
129 acquisition software (Pico Technology Ltd., Cambridge, UK). Samples were flipped over
130 halfway (7.5 min) during the browning period. In a separate study, the same methodology and
131 antimicrobial treatments described above was repeated, but this time, the treated, breaded
132 samples were browned by deep frying (190°C, 15 s) in 3 liters of vegetable oil (Pure Wesson

133 Vegetable Oil, ConAgra Foods, Omaha, NE), using a Presto Digital Pro Fry deep fryer (Eau
134 Claire, WI). The temperature of the vegetable oil in the deep fryer and the geometric center of
135 products was continuously monitored and recorded at 1 s intervals during browning, as described
136 above. After oven or fryer browning, products were allowed to cool and were then individually
137 packaged in double zipper polyethylene bags (Ziploc, S.C. Johnson, Racine, WI) and stored at -
138 20°C for 7 days.

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140 **Microbiological and physicochemical analyses.** Samples were analyzed for microbial
141 counts at four points of the process, specifically, (1) after inoculation, (2) after grinding (i.e.,
142 approximately 15 min after antimicrobial addition), (3) after browning (i.e., within 2 to 3 min
143 after removal of the products from the oven or fryer), and (4) after 7 days of frozen (-20°C)
144 storage. For sampling points 1 and 2, 25 g samples were analyzed, whereas for analysis points 3
145 and 4, samples were comprised of the entire 150 g breaded chicken product. Frozen samples
146 (sampling point 4) were thawed for 15-18 h at 4°C before microbial analysis. Samples (25 or 150
147 g) were placed in a Whirl-Pak filter bag (Nasco, Modesto, CA), to which diluent (0.85% NaCl
148 and 0.1% peptone [Difco, Becton Dickinson]) was added at a 1:1 ratio of sample weight (g) to
149 volume (ml) of diluent. The samples were homogenized (Masticator, IUL Instruments,
150 Barcelona, Spain) for 2 min, serially diluted in 0.1% buffered peptone water (Difco, Becton
151 Dickinson), and surface-plated for *Salmonella* counts on XLD agar, and total bacterial counts on
152 tryptic soy agar (Acumedia) supplemented with 0.1% sodium pyruvate (Fisher Scientific,
153 Pittsburgh, PA) (TSAP). Colonies were enumerated after incubation of plates at 35°C for 24 h
154 (XLD agar) or 25°C for 72 h (TSAP). The detection limit of the analysis was 0.3 log CFU/g.
155 Uninoculated, raw chicken breast meat samples were also analyzed to determine the natural

156 microbial contamination level of the chicken meat used to prepare the surface-browned,
157 uncooked, breaded chicken products.

158 After microbial analysis, pH measurements were taken of the sample homogenates with a
159 Denver Instruments (Arvada, CO) pH meter fitted with a glass electrode. Also, water activity
160 measurements (AquaLab model series 3, Decagon Devices, Pullman, WA) were taken of the
161 surface-browned, breaded chicken products before frozen storage.

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163 **Statistical analysis.** At each sampling point, three samples per treatment were analyzed in
164 each of two repetitions of each product type (i.e., oven- or fryer-browned). The pH, water
165 activity, and microbiological (converted to log CFU/g) data were analyzed with the PROC
166 MIXED procedures of SAS (version 9.3, SAS Institute Inc., Cary, NC) with independent
167 variables including antimicrobial treatment, sampling point, and their interaction. Means were
168 separated with the Tukey-adjusted procedure and were considered significant when P-values
169 were less than 0.05.

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171 **RESULTS AND DISCUSSION**

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173 **Physicochemical properties of products.** The pH values of untreated control surface-
174 browned chicken samples after frozen storage were 6.04 (oven-browned) and 6.19 (fryer-
175 browned) (Tables 1 and 2). Treatment of the chicken breast meat with CAA (0.5 and 1.0%),
176 CAR (0.5%), or POL (0.125 and 0.25%) had, in some cases, statistically significant ($P < 0.05$)
177 effects on the pH of the final products (i.e., sampling point 4). However, in all these cases, the
178 actual difference in pH values of these treatments and the pH of the corresponding untreated

179 control in each study was small (0.09 to 0.30 pH units; Tables 1 and 2). Water activities of
180 untreated surface-browned chicken samples were 0.978 (oven-browned) and 0.977 (fryer-
181 browned), and for samples treated with antimicrobials water activities ranged from 0.975 (0.25%
182 POL) to 0.980 (0.5% CAR) in oven-browned products, and 0.976 (1.0% CAA) to 0.979 (0.5%
183 CAR) in fryer-browned samples (Tables 1 and 2).

184

185 **Microbial counts during manufacture and after frozen storage of products.** Total
186 bacterial counts of the uninoculated, raw chicken breast meat used to prepare the products were
187 4.7 ± 0.8 to 4.9 ± 0.5 log CFU/g, while hydrogen sulfide-producing populations, on XLD agar,
188 were not detected (<0.3 log CFU/g) in any of the uninoculated samples (data not shown in
189 tables).

190 Initial inoculated *Salmonella* counts for all treatments ranged from 4.8 to 5.0 log CFU/g, and
191 initial total bacterial counts ranged from 5.0 to 5.5 log CFU/g (Tables 3 and 4). As previously
192 described, between sampling point 1 (i.e., after inoculation) and sampling point 2, inoculated
193 chicken meat portions were treated with an antimicrobial solution or distilled water, salt and
194 phosphate were added and the resulting mixture was ground. During the approximately 15 min
195 period between sampling points 1 and 2, initial pathogen counts of CAA-, CAR-, and POL-
196 treated samples were reduced by 1.8 to >4.4 , 3.1 to >4.0 , and 0.3 to 0.5 log CFU/g, respectively,
197 irrespective of antimicrobial concentration (Tables 3 and 4). However, only CAA- and CAR-
198 treated samples had significantly ($P<0.05$) lower counts compared to the untreated control at
199 sampling point 2; thus, these antimicrobials and tested concentrations effectively reduced
200 *Salmonella* contamination in the raw, ground chicken breast mixture. CAA is a generally
201 recognized as safe (CFR 184.1025) food-grade chemical and has been found to be effective

202 against *Salmonella* in sterile chicken cecal contents (23) and on alfalfa seeds (7). Use of 0.7 or
203 1.0% CAA as a feed supplement was also reported to reduce *Salmonella* colonization of day-old
204 chicks (15). CAR is one of the main components of oregano essential oil and its antimicrobial
205 properties against *Salmonella* and other foodborne pathogens, in laboratory media and various
206 food products, are well-documented (4, 5, 24). Addition of 0.6 or 0.9% oregano essential oil to
207 ground sheep meat resulted in significant reductions of *Salmonella* Enteritidis populations during
208 a 12-day storage period at 4 or 10°C, and furthermore, treated ground meat samples were found
209 organoleptically acceptable by a trained sensory panel (12). Further studies are needed to
210 determine the organoleptic acceptability of CAA and CAR in breaded chicken products.

211 The average maximum temperature of the geometric center of samples from all treatments
212 was 44.1±3.0°C during the 15 min oven browning period (Fig. 1), and 35.3±1.0°C during the 15
213 s deep fryer browning period (Fig. 2). End-point geometric center temperatures for the individual
214 product treatments and two surface browning methods are shown in Table 5. Irrespective of
215 antimicrobial treatment, *Salmonella* counts of samples analyzed after fryer browning (sampling
216 point 3) were not ($P \geq 0.05$) different than those of samples analyzed after grinding (sampling
217 point 2) (Table 4). Similar findings were obtained for oven-browned products except for samples
218 treated with 0.5% CAA or POL (0.125 and 0.25%) (Table 3). For these treatments, pathogen
219 counts after oven browning were 0.4 (0.125 and 0.25% POL) and at least 1.5 (0.5% CAA) log
220 CFU/g lower ($P < 0.05$) than those obtained at sampling point 2.

221 Pathogen counts of samples analyzed after frozen storage (-20°C, 7 days; sampling point 4)
222 were numerically, and in most cases, significantly ($P < 0.05$) lower than those of samples analyzed
223 after oven or fryer browning (sampling point 3), regardless of antimicrobial treatment (Tables 3
224 and 4). Overall, compared to initial populations (sampling point 1), total reductions of inoculated

225 *Salmonella* in untreated control oven- or fryer-browned products after frozen storage were 1.2
226 and 0.8 log CFU/g, respectively, while total bacterial populations were reduced by 0.7 and 0.5
227 log CFU/g, respectively (Tables 3 and 4). Survival of *Salmonella* during frozen storage of
228 breaded chicken products has been previously reported by Dominguez and Schaffner (9).
229 Specifically, *Salmonella* populations, as recovered on XLT-4 agar, in fully-cooked breaded
230 chicken nuggets or uncooked breaded chicken strips inoculated (4-5 log CFU/g) after
231 manufacture, decreased by approximately 1 log CFU/g after 16 weeks of storage at -20°C (9). In
232 the present study, total pathogen reductions for samples treated with CAA (0.5 or 1.0%), CAR
233 (0.3 or 0.5%) or POL (0.125 or 0.25%) were 4.1 to >4.5, >4.0, and 1.5 to 1.6 log CFU/g,
234 respectively, after frozen storage of oven-browned samples (Table 3), and 3.3 to >4.3, 4.1 to
235 >4.7, and 1.1 log CFU/g, respectively, after frozen storage of fryer-browned samples (Table 4).
236 In particular, treatment of samples with 1.0% CAA (oven-browned) or 0.5% CAR (oven- or
237 fryer-browned) reduced initial *Salmonella* counts to below the detection limit (<0.3 log CFU/g)
238 in stored frozen products. Compared to the untreated control in each study, all antimicrobials and
239 concentrations tested, except POL (0.125 or 0.25%), significantly (P<0.05) reduced *Salmonella*
240 and total bacterial counts in the final, oven- or fryer-browned, frozen product. *Salmonella* counts
241 of products treated with 0.125 or 0.25% POL were 0.2 to 0.4 log CFU/g lower (P≥0.05) than
242 those of the untreated control after frozen storage. Based on previous reports (6, 11, 13) on the
243 antimicrobial activity of POL, alone or in combination with other antimicrobials, further studies
244 are warranted to determine the effectiveness against *Salmonella* of POL added individually,
245 possibly at higher concentrations than those tested in this study and/or in combination with other
246 antimicrobials, in breaded chicken products.

247 In summary, this study demonstrated the potential of caprylic acid and carvacrol to reduce
248 *Salmonella* contamination in raw chicken meat portions intended for the manufacture of surface-
249 browned, frozen, breaded chicken products. Further work is needed to determine minimum
250 effective concentration levels of these antimicrobials, used individually or in combinations,
251 against *Salmonella* contamination in raw chicken portions. In such future studies, ϵ -polylysine
252 should not be neglected as it could also be effective when used at higher concentrations or in
253 combination with other antimicrobials. Until antimicrobial interventions are used or other
254 preventive control measures are taken by the industry, appropriate labeling (18, 22) on the
255 package of surface-browned, uncooked, frozen breaded chicken products and consumer
256 education about the hazards associated with consumption of raw or undercooked chicken
257 products, are the only means to lower the risk of salmonellosis from these types of products.

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263

264 REFERENCES

265

- 266 1. Benli, H., M. X. Sanchez-Plata, and J. T. Keeton. 2011. Efficacy of ϵ -polylysine, lauric
267 arginate, or acidic calcium sulfate applied sequentially for *Salmonella* reduction on
268 membrane filters and chicken carcasses. *J. Food Prot.* 74:743-750.

- 269 2. Bucher, O., J.-Y. D'Aoust, and R. A. Holley. 2008. Thermal resistance of *Salmonella*
270 serovars isolated from raw, frozen chicken nuggets/strips, nugget meat and pelleted broiler
271 feed. *Int. J. Food Microbiol.* 124:195-198.
- 272 3. Bucher, O., R. A. Holley, R. Ahmed, H. Tabor, C. Nadon, L. K. Ng, and J.-Y. D'Aoust.
273 2007. Occurrence and characterization of *Salmonella* from chicken nuggets, strips, and
274 pelleted broiler feed. *J. Food Prot.* 70:2251-2258.
- 275 4. Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods-
276 a review. *Int. J. Food Microbiol.* 94:223-253.
- 277 5. Burt, S. A., M. J. Fledderman, H. P. Haagsman, F. van Knapen, and E. J. A. Veldhuizen.
278 2007. Inhibition of *Salmonella enterica* serotype Enteritidis on agar and raw chicken by
279 carvacrol vapour. *Int. J. Food Microbiol.* 119:346-350.
- 280 6. Chang, S.-S., W.-Y. W. Lu, S.-H. Park, and D.-H. Kang. 2010. Control of foodborne
281 pathogens on ready-to-eat roast beef slurry by ϵ -polylysine. *Int. J. Food Microbiol.* 141:236-
282 241.
- 283 7. Chang, S.-S., M. Redondo-Solano, and H. Thippareddi. 2010. Inactivation of *Escherichia*
284 *coli* O157:H7 and *Salmonella* spp. on alfalfa seeds by caprylic acid and monocaprylin. *Int. J.*
285 *Food Microbiol.* 144:141-146.
- 286 8. Currie, A., L. MacDougall, J. Aramini, C. Gaulin, R. Ahmed, and S. Isaacs. 2005. Frozen
287 chicken nuggets and strips and eggs are leading risk factors for *Salmonella* Heidelberg
288 infections in Canada. *Epidemiol. Infect.* 133:809-816.
- 289 9. Dominguez, S. A., and D. W. Schaffner. 2009. Survival of *Salmonella* in processed chicken
290 products during frozen storage. *J. Food Prot.* 72:2088-2092.

- 291 10. Eglezos, S., G. A. Dykes, B. Huang, N. Fegan, and E. Stuttard. 2008. Bacteriological profile
292 of raw, frozen chicken nuggets. *J. Food Prot.* 71:613-615.
- 293 11. Geornaras, I., Y. Yoon, K. E. Belk, G. C. Smith, and J. N. Sofos. 2007. Antimicrobial
294 activity of ϵ -polylysine against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and
295 *Listeria monocytogenes* in various food extracts. *J. Food Sci.*72:M330-M334.
- 296 12. Govaris, A., N. Solomakos, A. Pexara, and P. S. Chatzopoulou. 2010. The antimicrobial
297 effect of oregano essential oil, nisin and their combination against *Salmonella* Enteritidis in
298 minced sheep meat during refrigerated storage. *Int. J. Food Microbiol.* 137:175-180.
- 299 13. Jung, Y. J., K. J. Min, and K. S. Yoon. 2009. Responses of acid-stressed *Salmonella*
300 Typhimurium in broth and chicken patties to subsequent antimicrobial stress with ϵ -
301 polylysine and combined potassium lactate and sodium diacetate. *Food Microbiol.* 26:467-
302 474.
- 303 14. Kenny, B., R. Hall, and S. Cameron. 1999. Consumer attitudes and behaviours - key risk
304 factors in an outbreak of *Salmonella* Typhimurium phage type 12 infection sourced to
305 chicken nuggets. *Aust. N. Z. J. Public Health* 23:164-167.
- 306 15. Kollanoor Johny A., S. Ananda Baskaran, A. S. Charles, M. A. Roshni Amalaradjou, M. J.
307 Darre, M. I. Khan, T. A. Hoagland, D. T. Schreiber, A. M. Donoghue, D. J. Donoghue, and
308 K. Venkitanarayanan. 2009. Prophylactic supplementation of caprylic acid in feed reduces
309 *Salmonella* Enteritidis colonization in commercial broiler chicks. *J. Food Prot.* 72:722-727.
- 310 16. Loretz, M., R. Stephan, and C. Zweifel. 2010. Antimicrobial activity of decontamination
311 treatments for poultry carcasses: a literature survey. *Food Control* 21:791-804.

- 312 17. MacDougall, L., M. Fyfe, L. McIntyre, A. Paccagnella, K. Cordner, A. Kerr, and J. Aramini.
313 2004. Frozen chicken nuggets and strips - a newly identified risk factor for *Salmonella*
314 Heidelberg infection in British Columbia, Canada. *J. Food Prot.* 67:1111-1115.
- 315 18. NACMCF (National Advisory Committee on Microbiological Criteria for Foods). 2007.
316 Response to the questions posed by the Food Safety and Inspection Service regarding
317 consumer guidelines for the safe cooking of poultry products. *J. Food Prot.* 70:251-260.
- 318 19. Owens, C. M. 2001. Coated poultry products, p. 227-242. In A. R. Sams (ed.), Poultry meat
319 processing. CRC Press, Boca Raton, FL.
- 320 20. Phebus, R., D. Powell, and H. Thippareddi. 2009. Beyond intent: assessment and validation
321 of on-package handling and cooking instructions for uncooked, breaded meat and poultry
322 products to promote consumer practices that reduce foodborne illness risks. Available at:
323 <http://www.amif.org/ht/d/sp/i/26883/pid/26883#Salmonella>. Accessed 31 October 2011.
- 324 21. Smith, K. E., C. Medus, S. D. Meyer, D. J. Boxrud, F. Leano, C. W. Hedberg, K. Elfering, C.
325 Braymen, J. B. Bender, and R. N. Danila. 2008. Outbreaks of salmonellosis in Minnesota
326 (1998 through 2006) associated with frozen, microwaveable, breaded, stuffed chicken
327 products. *J. Food Prot.* 71:2153-2160.
- 328 22. U. S. Department of Agriculture, Food Safety and Inspection Service. 2007. Labeling policy
329 guidance - uncooked, breaded, boneless poultry products. Available at:
330 [http://www.fsis.usda.gov/PDF/Labeling_Policy_Guidance_Uncooked_Breaded_Boneless_Po](http://www.fsis.usda.gov/PDF/Labeling_Policy_Guidance_Uncooked_Breaded_Boneless_Poultry_Products.pdf)
331 [ultry_Products.pdf](http://www.fsis.usda.gov/PDF/Labeling_Policy_Guidance_Uncooked_Breaded_Boneless_Poultry_Products.pdf). Accessed 31 October 2011.
- 332 23. Vasudevan, P., P. Marek, M. K. M. Nair, T. Annamalai, M. Darre, M. Khan, and K.
333 Venkitanarayanan. 2005. In vitro inactivation of *Salmonella* Enteritidis in autoclaved chicken
334 cecal contents by caprylic acid. *J. Appl. Poult. Res.* 14:122-125.

335 24. Zhou, F., B. Ji, H. Zhang, H. Jiang, Z. Yang, J. Li, Y. Ren, and W. Yan. 2007. Synergistic
336 effect of thymol and carvacrol combined with chelators and organic acids against *Salmonella*
337 Typhimurium. *J. Food Prot.* 70:1704-1709.
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FIGURE LEGENDS

340

341 FIGURE 1. Changes in the temperature of the oven chamber (■) and the geometric center of
342 samples (▲) during oven browning of breaded chicken products.

343

344 FIGURE 2. Changes in the temperature of the vegetable oil in the deep fryer (■) and the
345 geometric center of samples (▲) during fryer browning of breaded chicken products.

346

347 TABLE 1. The effect of various concentrations of caprylic acid, carvacrol, and ϵ -polylysine on the pH values (mean \pm standard
 348 deviation) of samples at different stages during the manufacture of a frozen, not-ready-to-eat breaded chicken product surface-
 349 browned in an oven (208°C, 15 min), and on the water activity values (mean \pm standard deviation) of the browned breaded chicken
 350 products.

Treatment	pH				Water activity
	After inoculation	After grinding	After baking	After frozen storage	After baking
Distilled water (control)	5.87 \pm 0.04 aC	5.98 \pm 0.03 cB	6.04 \pm 0.04 bA	6.04 \pm 0.02 bA	0.978 \pm 0.000 b
Caprylic acid (0.5%)	5.85 \pm 0.08 aB	5.81 \pm 0.02 dB	5.95 \pm 0.02 cA	5.95 \pm 0.02 cA	0.977 \pm 0.001 bc
Caprylic acid (1.0%)	5.83 \pm 0.06 aA	5.66 \pm 0.01 eB	5.78 \pm 0.04 dA	5.77 \pm 0.04 dA	0.976 \pm 0.000 cd
Carvacrol (0.3%)	5.87 \pm 0.11 aB	6.01 \pm 0.05 cA	6.09 \pm 0.06 bA	6.10 \pm 0.05 bA	0.978 \pm 0.001 b
Carvacrol (0.5%)	5.82 \pm 0.05 aC	6.01 \pm 0.03 cB	6.09 \pm 0.02 bA	6.09 \pm 0.02 bA	0.980 \pm 0.001 a
ϵ -Polylysine (0.125%)	5.88 \pm 0.04 aC	6.13 \pm 0.03 bB	6.18 \pm 0.02 aA	6.18 \pm 0.02 aA	0.977 \pm 0.001 b
ϵ -Polylysine (0.25%)	5.92 \pm 0.07 aB	6.22 \pm 0.01 aA	6.20 \pm 0.06 aA	6.20 \pm 0.05 aA	0.975 \pm 0.001 d

351 Within a column, means lacking a common lowercase letter are different (P<0.05).

352 Within a row and pH values, means lacking a common uppercase letter are different (P<0.05).

353

354 TABLE 2. The effect of various concentrations of caprylic acid, carvacrol, and ϵ -polylysine on the pH values (mean \pm standard
 355 deviation) of samples at different stages during the manufacture of a frozen, not-ready-to-eat breaded chicken product surface-
 356 browned in a deep fryer (190°C, 15 s), and on the water activity values (mean \pm standard deviation) of the browned breaded chicken
 357 products.

Treatment	pH				Water activity
	After inoculation	After grinding	After frying	After frozen storage	After frying
Distilled water (control)	5.88 \pm 0.11 abB	6.11 \pm 0.08 bA	6.10 \pm 0.09 aA	6.19 \pm 0.10 abA	0.977 \pm 0.001 b
Caprylic acid (0.5%)	5.94 \pm 0.09 abA	5.90 \pm 0.06 dA	5.94 \pm 0.06 bcA	6.02 \pm 0.10 cdA	0.977 \pm 0.001 bc
Caprylic acid (1.0%)	5.90 \pm 0.07 abA	5.68 \pm 0.02 eB	5.86 \pm 0.04 cA	5.89 \pm 0.06 dA	0.976 \pm 0.001 c
Carvacrol (0.3%)	5.95 \pm 0.07 abB	6.01 \pm 0.02 cB	6.00 \pm 0.03 bB	6.11 \pm 0.02 bcA	0.977 \pm 0.000 bc
Carvacrol (0.5%)	5.81 \pm 0.05 bC	5.95 \pm 0.02 cdB	5.97 \pm 0.01 bB	6.04 \pm 0.01 cA	0.979 \pm 0.001 a
ϵ -Polylysine (0.125%)	5.96 \pm 0.09 aB	6.16 \pm 0.01 bA	6.11 \pm 0.05 aA	6.20 \pm 0.06 abA	0.977 \pm 0.000 bc
ϵ -Polylysine (0.25%)	5.97 \pm 0.07 aB	6.27 \pm 0.06 aA	6.19 \pm 0.06 aA	6.30 \pm 0.10 aA	0.977 \pm 0.001 bc

358 Within a column, means lacking a common lowercase letter are different (P<0.05).

359 Within a row and pH values, means lacking a common uppercase letter are different (P<0.05).

360

361 TABLE 3. The effect of various concentrations of caprylic acid, carvacrol, and ϵ -polylysine on *Salmonella* and total bacterial counts
 362 (mean \pm standard deviation; log CFU/g) at different stages during the manufacture of a frozen, not-ready-to-eat breaded chicken
 363 product surface-browned in an oven (208°C, 15 min).

Treatment	<i>Salmonella</i> counts				Total bacterial counts			
	After inoculation	After grinding	After baking	After frozen storage	After inoculation	After grinding	After baking	After frozen storage
Distilled water (control)	4.8 \pm 0.1 aA	4.6 \pm 0.1 aAB	4.4 \pm 0.2 aB	3.6 \pm 0.2 aC	5.4 \pm 0.4 aA	5.2 \pm 0.4 aAB	4.9 \pm 0.2 aAB	4.7 \pm 0.4 aB
Caprylic acid (0.5%)	4.9 \pm 0.2 aA	2.9 \pm 0.2 bB	<1.4 \pm 0.4 bcC	0.8 \pm 0.4 bcD	5.5 \pm 0.5 aA	3.3 \pm 0.4 bB	2.6 \pm 0.2 bC	2.4 \pm 0.3 bC
Caprylic acid (1.0%)	4.8 \pm 0.2 aA	<0.8 \pm 0.5 cB	<0.8 \pm 0.5 cB	<0.3 ¹ cB	5.0 \pm 0.2 aA	<1.4 \pm 1.2 cB	<1.3 \pm 1.1 cB	<0.8 \pm 0.6 cB
Carvacrol (0.3%)	4.9 \pm 0.1 aA	<1.4 \pm 1.0 cBC	1.8 \pm 0.3 bB	<0.9 \pm 0.4 bC	5.1 \pm 0.2 aA	2.8 \pm 0.3 bB	2.7 \pm 0.1 bB	2.3 \pm 0.1 bC
Carvacrol (0.5%)	4.9 \pm 0.2 aA	<0.9 \pm 0.5 cB	<0.8 \pm 0.5 cB	<0.3 cB	5.4 \pm 0.4 aA	2.5 \pm 0.7 bB	2.7 \pm 0.4 bB	3.0 \pm 1.1 bB
ϵ -Polylysine (0.125%)	4.9 \pm 0.1 aA	4.4 \pm 0.2 aB	4.0 \pm 0.1 aC	3.4 \pm 0.2 aD	5.4 \pm 0.3 aA	5.0 \pm 0.1 aB	4.7 \pm 0.3 aC	4.1 \pm 0.0 aD
ϵ -Polylysine (0.25%)	4.8 \pm 0.2 aA	4.3 \pm 0.1 aB	3.9 \pm 0.1 aC	3.2 \pm 0.4 aD	5.3 \pm 0.3 aA	4.9 \pm 0.3 aAB	4.6 \pm 0.2 aBC	4.5 \pm 0.3 aC

364 ¹Detection limit: 0.3 log CFU/g.

365 Within a column, means lacking a common lowercase letter are different (P<0.05).

366 Within a row and within each microbial count (*Salmonella* or total bacterial counts), means lacking a common uppercase letter are
 367 different (P<0.05).

368

369 TABLE 4. The effect of various concentrations of caprylic acid, carvacrol, and ϵ -polylysine on *Salmonella* and total bacterial counts
 370 (mean \pm standard deviation; log CFU/g) at different stages during the manufacture of a frozen, not-ready-to-eat breaded chicken
 371 product surface-browned in a deep fryer (190°C, 15 s).

Treatment	<i>Salmonella</i> counts				Total bacterial counts			
	After inoculation	After grinding	After frying	After frozen storage	After inoculation	After grinding	After frying	After frozen storage
Distilled water (control)	4.9 \pm 0.2 aA	4.7 \pm 0.1 aAB	4.6 \pm 0.1 aB	4.1 \pm 0.3 aC	5.3 \pm 0.3 aA	5.1 \pm 0.1 aAB	4.9 \pm 0.2 aAB	4.8 \pm 0.3 aB
Caprylic acid (0.5%)	4.9 \pm 0.1 aA	3.1 \pm 0.1 bB	2.7 \pm 0.2 bB	1.6 \pm 0.5 bC	5.3 \pm 0.3 aA	3.7 \pm 0.0 cB	3.5 \pm 0.2 bB	3.5 \pm 0.5 bB
Caprylic acid (1.0%)	4.8 \pm 0.1 aA	<0.4 \pm 0.1 eB	<0.8 \pm 0.4 cB	<0.5 \pm 0.4 cdB	5.3 \pm 0.2 aA	2.1 \pm 0.2 eB	2.0 \pm 0.5 dB	2.4 \pm 0.6 cB
Carvacrol (0.3%)	5.0 \pm 0.1 aA	1.9 \pm 0.5 cB	2.3 \pm 0.4 bB	0.9 \pm 0.4 cC	5.2 \pm 0.1 aA	2.8 \pm 0.1 dB	2.6 \pm 0.2 cB	2.6 \pm 0.8 cB
Carvacrol (0.5%)	5.0 \pm 0.1 aA	<1.1 \pm 0.6 dB	1.3 \pm 0.4 cB	<0.3 ¹ dC	5.0 \pm 0.1 aA	2.0 \pm 0.2 eB	2.1 \pm 0.1 dB	1.5 \pm 0.1 dC
ϵ -Polylysine (0.125%)	4.9 \pm 0.1 aA	4.6 \pm 0.1 aAB	4.2 \pm 0.5 aBC	3.8 \pm 0.2 aC	5.2 \pm 0.2 aA	4.9 \pm 0.1 abB	4.9 \pm 0.1 aB	4.8 \pm 0.3 aB
ϵ -Polylysine (0.25%)	4.9 \pm 0.2 aA	4.4 \pm 0.2 aB	4.4 \pm 0.1 aB	3.8 \pm 0.1 aC	5.3 \pm 0.3 aA	4.8 \pm 0.1 bB	4.8 \pm 0.1 aB	4.7 \pm 0.2 aB

372 ¹Detection limit: 0.3 log CFU/g.

373 Within a column, means lacking a common lowercase letter are different (P<0.05).

374 Within a row and within each microbial count (*Salmonella* or total bacterial counts), means lacking a common uppercase letter are

375 different (P<0.05).

376 TABLE 5. End-point temperatures (mean±standard deviation) of the geometric center of breaded
 377 chicken products surface-browned in an oven (208°C, 15 min) or deep fryer (190°C, 15 s).

Treatment	Temperature (°C)	
	Oven-browned	Fryer-browned
Distilled water (control)	42.4±1.5	35.9±0.2
Caprylic acid (0.5%)	43.1±1.1	36.4±0.8
Caprylic acid (1.0%)	49.1±6.3	35.5±0.8
Carvacrol (0.3%)	43.5±1.9	34.9±0.1
Carvacrol (0.5%)	46.2±3.3	34.9±2.1
ε-Polylysine (0.125%)	42.9±2.0	34.6±0.7
ε-Polylysine (0.25%)	43.1±0.2	35.2±1.5

378

379

FIGURE 1

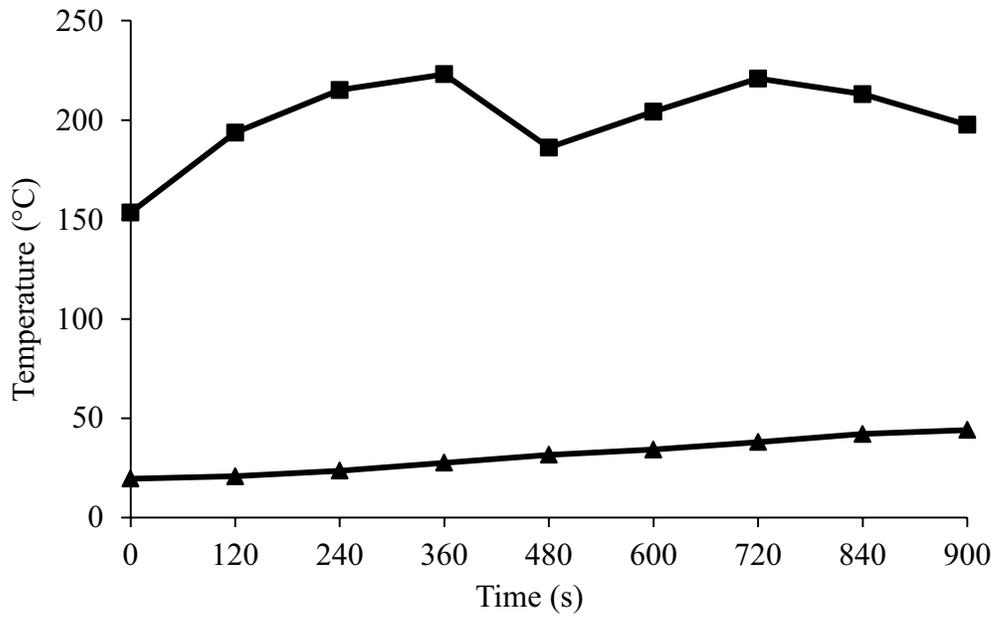


FIGURE 2

