

1 **Research Paper**

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

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5 **Antimicrobials for Reduction of *Salmonella* Contamination in Uncooked, Surface-Browned**

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**Breaded Chicken Products**

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

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## ABSTRACT

19  
20 Surface-browned but uncooked, frozen breaded chicken products have been associated with  
21 salmonellosis outbreaks due to inadequate or no cooking of the products before consumption.  
22 This study evaluated the effect of three antimicrobials against *Salmonella* during manufacture of  
23 a surface-browned, uncooked, frozen breaded chicken meat product. Fresh chicken breast meat  
24 portions (5 × 5 × 5 cm) were inoculated (4-5 log CFU/g) with *Salmonella* and mixed with  
25 caprylic acid (CAA; 0.5 and 1.0%), carvacrol (CAR; 0.3 and 0.5%), ε-polylysine (POL; 0.125  
26 and 0.25%) or distilled water (control). Sodium chloride (1.2%) and sodium tripolyphosphate  
27 (0.3%) were added to all treatments followed by grinding of the mixtures (5% total moisture  
28 enhancement level) and forming into 9 × 5 × 3 cm portions. The products were breaded and  
29 surface-browned by oven baking (208°C, 15 min) or deep frying in vegetable oil (190°C, 15 s),  
30 packaged in polyethylene bags, and stored at -20°C (7 days). Total reductions of inoculated  
31 *Salmonella* in untreated control oven- or fryer-browned products after frozen storage were 1.2  
32 and 0.8 log CFU/g, respectively. In comparison, treatment with CAA, CAR or POL reduced  
33 initial pathogen counts by 3.3 to >4.5, 4.1 to >4.7, and 1.1 to 1.6 log CFU/g, respectively,  
34 irrespective of antimicrobial concentration and browning method. Treatment with 1.0% CAA  
35 (oven-browned) or 0.5% CAR (oven/fryer-browned) reduced *Salmonella* to non-detectable levels  
36 (<0.3 log CFU/g) in stored frozen products. These data may be useful in the development of  
37 suitable antimicrobial treatments to reduce the risk of *Salmonella* contamination in surface-  
38 browned, uncooked, frozen breaded chicken products.

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## HIGHLIGHTS

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- Caprylic acid and carvacrol decreased *Salmonella* populations in uncooked chicken.

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- Addition of  $\epsilon$ -polylysine did not affect *Salmonella* populations.

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- Safe handling of uncooked chicken remains critical to minimize foodborne illness.

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

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

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

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

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## 80 MATERIALS AND METHODS

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82 **Bacterial strains and inoculum preparation.** The inoculum was comprised of seven  
83 *Salmonella* isolates of chicken or turkey origin (kindly provided by Dr. Vijay Juneja, Microbial  
84 Food Safety Research Unit, ERRC-ARS-USDA, Wyndmoor, PA), and included *Salmonella*  
85 Hadar FSIS 064/VJS6 (chicken), *Salmonella* Hadar FSIS MF61777/VJS19 (turkey), *Salmonella*  
86 Kentucky FSIS 044/VJS2 (chicken), *Salmonella* Kentucky FSIS 062/VJS1 (chicken), *Salmonella*  
87 Muenster FSIS MF61976/VJS15 (turkey), *Salmonella* Reading FSIS MF58210/VJS17 (turkey),  
88 and *Salmonella* Thompson FSIS 132/VJS7 (chicken). These *Salmonella* serotype strains formed  
89 colonies with black centers on xylose lysine deoxycholate (XLD) agar (Acumedia, Lansing, MI)  
90 indicating hydrogen sulfide production. The strains were individually cultured and subcultured in  
91 10 ml tryptic soy broth (Difco, Becton Dickinson, Sparks, MD) for 18-24 h at 35°C. The cell

92 cultures were then combined, harvested by centrifugation (4,629×g, 15 min, 4°C; Eppendorf  
93 model 5810 R, Brinkmann Instruments Inc., Westbury, NY) and washed twice in 10 ml  
94 phosphate-buffered saline (PBS, pH 7.4; 0.2 g/liter KH<sub>2</sub>PO<sub>4</sub>, 1.5 g/liter Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O,  
95 8.0 g/liter NaCl, and 0.2 g/liter KCl). The washed cell pellet was resuspended in 70 ml PBS and  
96 further diluted, in PBS, to a concentration of 6-7 log CFU/ml.

97  
98 **Inoculation, treatment, product preparation, and storage.** Fresh, boneless, skinless  
99 chicken breasts were purchased directly from a poultry processing facility in Colorado. If not  
100 used within 24 h, the chicken breasts were vacuum-packaged and stored at -20°C. When needed,  
101 they were thawed at 4°C for approximately 48 h before use. The chicken breast meat was cut into  
102 pieces (approximately 5 × 5 × 5 cm), and batches of 2 kg were inoculated with 20 ml of the  
103 *Salmonella* inoculum to a target level of 4-5 log CFU/g. The chicken meat and inoculum were  
104 thoroughly mixed for 2 min using a KitchenAid Professional 600™ mixer (St. Joseph, MI) at a  
105 speed setting of “stir”, and then left to stand at 4°C for 30 min for bacterial cell attachment. The  
106 inoculated batches (2 kg) of chicken meat were then treated with 20 ml of one of the following  
107 treatments; as indicated, two concentrations of each antimicrobial were tested: (i) sterile distilled  
108 water (control), (ii) caprylic acid (CAA, 0.5 and 1.0% v/w; Fisher Scientific, Hampton, NH), (iii)  
109 carvacrol (CAR, 0.3 and 0.5% v/w; Acros Organics, Geel, Belgium), and (iv) ε-polylysine (POL,  
110 0.125 and 0.25% v/w; Chisso Corporation, Minamata, Japan). These antimicrobials were  
111 selected for evaluation based on results of a screening study (unpublished data) in which four  
112 concentration levels each of 10 antimicrobials (allyl isothiocyanate, caprylic acid, carvacrol,  
113 citric acid, grapefruit distilled terpene, malic acid, oregano oil, ε-polylysine, sodium citrate, and  
114 sodium lactate) were evaluated for antimicrobial effects against *Salmonella* inoculated on raw

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

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

138 Vegetable Oil, ConAgra Foods, Omaha, NE), using a Presto Digital Pro Fry deep fryer (Eau  
139 Claire, WI). The temperature of the vegetable oil in the deep fryer and the geometric center of  
140 products was continuously monitored and recorded at 1 s intervals during browning, as described  
141 above. After oven or fryer browning, products were allowed to cool and were then individually  
142 packaged in double zipper polyethylene bags (Ziploc, S.C. Johnson, Racine, WI) and stored at -  
143 20°C for 7 days.

144

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



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

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

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

175

## 176 **RESULTS AND DISCUSSION**

177 **Physicochemical properties of products.** The pH values of untreated control surface-  
178 browned chicken samples after frozen storage were 6.04 (oven-browned) and 6.19 (fryer-  
179 browned) (Tables 1 and 2). Treatment of the chicken breast meat with CAA (0.5 and 1.0%),  
180 CAR (0.5%), or POL (0.125 and 0.25%) had, in some cases, statistically significant ( $P < 0.05$ )  
181 effects on the pH of the final products (i.e., sampling point 4). However, in all these cases, the  
182 actual difference in pH values of these treatments and the pH of the corresponding untreated  
183 control in each study was small (0.09 to 0.30 pH units; Tables 1 and 2). Water activities of

184 untreated surface-browned chicken samples were 0.978 (oven-browned) and 0.977 (fryer-  
185 browned), and for samples treated with antimicrobials water activities ranged from 0.975 (0.25%  
186 POL) to 0.980 (0.5% CAR) in oven-browned products, and 0.976 (1.0% CAA) to 0.979 (0.5%  
187 CAR) in fryer-browned samples (Tables 1 and 2).

188

189 **Microbial counts during manufacture and after frozen storage of products.** Total  
190 bacterial counts of the uninoculated, raw chicken breast meat used to prepare the products were  
191  $4.7 \pm 0.8$  to  $4.9 \pm 0.5$  log CFU/g, while hydrogen sulfide-producing populations, on XLD agar,  
192 were not detected ( $<0.3$  log CFU/g) in any of the uninoculated samples (data not shown in  
193 tables).

194 Initial inoculated *Salmonella* counts for all treatments ranged from 4.8 to 5.0 log CFU/g, and  
195 initial total bacterial counts ranged from 5.0 to 5.5 log CFU/g (Tables 3 and 4). As previously  
196 described, between sampling point 1 (i.e., after inoculation) and sampling point 2, inoculated  
197 chicken meat portions were treated with an antimicrobial solution or distilled water, salt and  
198 phosphate were added and the resulting mixture was ground. During the approximately 15 min  
199 period between sampling points 1 and 2, initial pathogen counts of CAA-, CAR-, and POL-  
200 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,  
201 irrespective of antimicrobial concentration (Tables 3 and 4). However, only CAA- and CAR-  
202 treated samples had significantly ( $P < 0.05$ ) lower counts compared to the untreated control at  
203 sampling point 2; thus, these antimicrobials and tested concentrations effectively reduced  
204 *Salmonella* contamination in the raw, ground chicken breast mixture. CAA is a generally  
205 recognized as safe (CFR 184.1025) food-grade chemical and has been found to be effective  
206 against *Salmonella* in sterile chicken cecal contents (23) and on alfalfa seeds (7). Use of 0.7 or

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

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

225 Pathogen counts of samples analyzed after frozen storage ( $-20^\circ\text{C}$ , 7 days; sampling point 4)  
226 were numerically, and in most cases, significantly ( $P < 0.05$ ) lower than those of samples analyzed  
227 after oven or fryer browning (sampling point 3), regardless of antimicrobial treatment (Tables 3  
228 and 4). Overall, compared to initial populations (sampling point 1), total reductions of inoculated  
229 *Salmonella* in untreated control oven- or fryer-browned products after frozen storage were 1.2

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

251 In summary, this study demonstrated the potential of caprylic acid and carvacrol to reduce  
252 *Salmonella* contamination in raw chicken meat portions intended for the manufacture of surface-

253 browned, frozen, breaded chicken products. Further work is needed to determine minimum  
254 effective concentration levels of these antimicrobials, used individually or in combinations,  
255 against *Salmonella* contamination in raw chicken portions. In such future studies,  $\epsilon$ -polylysine  
256 should not be neglected as it could also be effective when used at higher concentrations or in  
257 combination with other antimicrobials. Until antimicrobial interventions are used or other  
258 preventive control measures are taken by the industry, appropriate labeling (18, 22) on the  
259 package of surface-browned, uncooked, frozen breaded chicken products and consumer  
260 education about the hazards associated with consumption of raw or undercooked chicken  
261 products, are the only means to lower the risk of salmonellosis from these types of products.

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#### SUPPLEMENTAL MATERIAL

268

Supplemental material associated with this article can be found online at: [URL to be  
269 completed by the publisher].

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#### REFERENCES

272

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

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

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

- 318 17. MacDougall, L., M. Fyfe, L. McIntyre, A. Paccagnella, K. Cordner, A. Kerr, and J. Aramini.  
319 2004. Frozen chicken nuggets and strips - a newly identified risk factor for *Salmonella*  
320 Heidelberg infection in British Columbia, Canada. *J. Food Prot.* 67:1111-1115.
- 321 18. National Advisory Committee on Microbiological Criteria for Foods. 2007. Response to the  
322 questions posed by the Food Safety and Inspection Service regarding consumer guidelines  
323 for the safe cooking of poultry products. *J. Food Prot.* 70:251-260.
- 324 19. Owens, C. M. 2001. Coated poultry products, p. 227-242. In A. R. Sams (ed.), Poultry meat  
325 processing. CRC Press, Boca Raton, FL.
- 326 20. Phebus, R., D. Powell, and H. Thippareddi. 2009. Beyond intent: assessment and validation  
327 of on-package handling and cooking instructions for uncooked, breaded meat and poultry  
328 products to promote consumer practices that reduce foodborne illness risks. Available at:  
329 <http://www.amif.org/ht/d/sp/i/26883/pid/26883#Salmonella>. Accessed 31 October 2011.
- 330 21. Smith, K. E., C. Medus, S. D. Meyer, D. J. Boxrud, F. Leano, C. W. Hedberg, K. Elfering, C.  
331 Braymen, J. B. Bender, and R. N. Danila. 2008. Outbreaks of salmonellosis in Minnesota  
332 (1998 through 2006) associated with frozen, microwaveable, breaded, stuffed chicken  
333 products. *J. Food Prot.* 71:2153-2160.
- 334 22. U.S. Department of Agriculture, Food Safety and Inspection Service. 2007. Labeling policy  
335 guidance - uncooked, breaded, boneless poultry products. Available at:  
336 [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)  
337 [ultry\\_Products.pdf](http://www.fsis.usda.gov/PDF/Labeling_Policy_Guidance_Uncooked_Breaded_Boneless_Poultry_Products.pdf). Accessed 31 October 2011.
- 338 23. Vasudevan, P., P. Marek, M. K. M. Nair, T. Annamalai, M. Darre, M. Khan, and K.  
339 Venkitanarayanan. 2005. In vitro inactivation of *Salmonella* Enteritidis in autoclaved chicken  
340 cecal contents by caprylic acid. *J. Appl. Poult. Res.* 14:122-125.



341 24. Zhou, F., B. Ji, H. Zhang, H. Jiang, Z. Yang, J. Li, Y. Ren, and W. Yan. 2007. Synergistic  
342 effect of thymol and carvacrol combined with chelators and organic acids against *Salmonella*  
343 Typhimurium. *J. Food Prot.* 70:1704-1709.  
344

345

## FIGURE LEGENDS

346

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

349

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

352

353 TABLE 1. The effect of various concentrations of caprylic acid, carvacrol, and  $\epsilon$ -polylysine on the pH values (mean $\pm$ standard  
 354 deviation) of samples at different stages during the manufacture of a frozen, not-ready-to-eat breaded chicken product surface-  
 355 browned in an oven (208°C, 15 min), and on the water activity values (mean $\pm$ standard deviation) of the browned breaded chicken  
 356 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
$\epsilon$ -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
$\epsilon$ -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

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

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

359

360 TABLE 2. The effect of various concentrations of caprylic acid, carvacrol, and  $\epsilon$ -polylysine on the pH values (mean $\pm$ standard  
 361 deviation) of samples at different stages during the manufacture of a frozen, not-ready-to-eat breaded chicken product surface-  
 362 browned in a deep fryer (190°C, 15 s), and on the water activity values (mean $\pm$ standard deviation) of the browned breaded chicken  
 363 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
$\epsilon$ -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
$\epsilon$ -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

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

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

366

367 TABLE 3. The effect of various concentrations of caprylic acid, carvacrol, and  $\epsilon$ -polylysine on *Salmonella* and total bacterial counts  
 368 (mean $\pm$ standard deviation; log CFU/g) at different stages during the manufacture of a frozen, not-ready-to-eat breaded chicken  
 369 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 <sup>1</sup> 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
$\epsilon$ -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
$\epsilon$ -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

370 <sup>1</sup>Detection limit: 0.3 log CFU/g.

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

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

374

375 TABLE 4. The effect of various concentrations of caprylic acid, carvacrol, and  $\epsilon$ -polylysine on *Salmonella* and total bacterial counts  
 376 (mean $\pm$ standard deviation; log CFU/g) at different stages during the manufacture of a frozen, not-ready-to-eat breaded chicken  
 377 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 <sup>1</sup> 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
$\epsilon$ -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
$\epsilon$ -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

378 <sup>1</sup>Detection limit: 0.3 log CFU/g.

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

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

381 different (P<0.05).

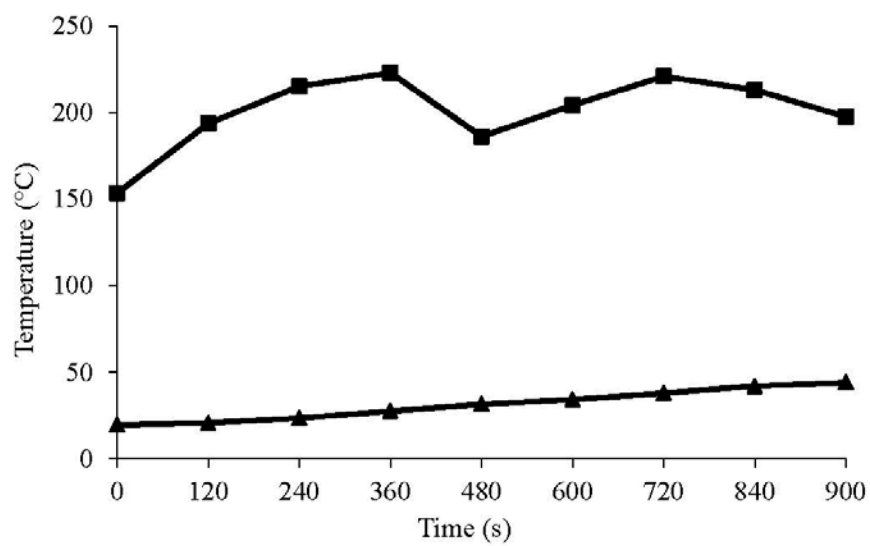
382 TABLE 5. End-point temperatures (mean±standard deviation) of the geometric center of breaded  
 383 chicken products surface-browned in an oven (208°C, 15 min) or deep fryer (190°C, 15 s).

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

384

385

**FIGURE 1**  
**JFP-11-492**  
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**FIGURE 2**  
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