FINAL REPORT

Development of an Intervention to Reduce the Likelihood of *Salmonella* Contamination in Raw Poultry Intended for use in the Manufacture of Frozen, Not-Ready-to-Eat Entrees

April, 2011

SUBMITTED TO:
AMERICAN MEAT INSTITUTE FOUNDATION

SUBMITTED BY:
CENTER FOR MEAT SAFETY & QUALITY
DEPARTMENT OF ANIMAL SCIENCES
COLORADO STATE UNIVERSITY, FORT COLLINS, CO
IN COLLABORATION WITH
PURAC NORTH AMERICA, LINCOLNSHIRE, IL, AND
PURAC BIOCHEM B.V., GORINCHEM, THE NETHERLANDS

GALATIOS D. MOSCHONAS
IFIGENIA GEORNARAS
JARRET D. STOPFORTH
DAMIEN WACH
DALE R. WOERNER
KEITH E. BELK
GARY C. SMITH
JOHN N. SOFOS

TABLE OF CONTENTS

I. EXECUTIVE SUMMARY 5
II. TECHNICAL ABSTRACT 6
III. INTRODUCTION 7
IV. OVERALL PROJECT GOAL 8
V. SUPPORTING OBJECTIVES 8
VI. MATERIALS AND METHODS 9
A. STUDY ADDRESSING OBJECTIVE 1 9
Study 1: Evaluation of four levels of 11 individual ingredients against *Salmonella* in diced, raw chicken breast 9
B. STUDIES ADDRESSING OBJECTIVES 2 AND 3 10
Study 2: The effect of various concentrations of caprylic acid, carvacrol, peracetic acid, and ε-polylysine on reductions of *Salmonella* contamination in frozen, oven-browned, breaded chicken products. 10
Study 3: The effect of various concentrations of caprylic acid, carvacrol, peracetic acid, and ε-polylysine on reductions of *Salmonella* contamination in frozen, fryer-browned, breaded chicken products. 12
Study 4: The effect of various concentrations of caprylic acid on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products 12
Study 5: The effect of various concentrations of carvacrol on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products 13
Study 6: The effect of various concentrations of ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products 13
Study 7: The effect of combinations of 0.25% caprylic acid, 0.3% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products. 13
Study 8: The effect of combinations of 0.125% caprylic acid, 0.15% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products. 14
Study 9: The effect of combinations of 0.03125% or 0.0625% caprylic acid, 0.0375% or 0.075% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products 14
C. STUDY ADDRESSING OBJECTIVE 4 15
Study 10: Evaluation of the organoleptic properties of fully cooked, breaded chicken products treated with selected antimicrobials. 15
D. ADDITIONAL STUDIES NOT INCLUDED IN THE ORIGINAL PROPOSAL 16
Study 11: The effect of product dimensions and surface browning method on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products treated with various antimicrobials. 16

Study 12: The effect of lauric arginate, and its combinations with caprylic acid, carvacrol, and/or ε-polylysine, on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products..... 17
VII. RESULTS..... 17
A. Study addressing Objective 1..... 17
Study 1: Evaluation of four levels of 11 individual ingredients against *Salmonella* in diced, raw chicken breast..... 17
B. Studies addressing Objectives 2 and 3..... 18
Study 2: The effect of various concentrations of caprylic acid, carvacrol, peracetic acid, and ε-polylysine on reductions of *Salmonella* contamination in frozen, oven-browned, breaded chicken products. 18
Study 3: The effect of various concentrations of caprylic acid, carvacrol, peracetic acid, and ε-polylysine on reductions of *Salmonella* contamination in frozen, fryer-browned, breaded chicken products. 19
Study 4: The effect of various concentrations of caprylic acid on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products..... 20
Study 5: The effect of various concentrations of carvacrol on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products..... 20
Study 6: The effect of various concentrations of ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products..... 21
Study 7: The effect of combinations of 0.25% caprylic acid, 0.3% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products. 21
Study 8: The effect of combinations of 0.125% caprylic acid, 0.15% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products. 22
Study 9: The effect of combinations of 0.03125% or 0.0625% caprylic acid, 0.0375% or 0.075% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products..... 23
C. Study addressing Objective 4..... 23
Study 10: Evaluation of the organoleptic properties of fully cooked, breaded chicken products treated with selected antimicrobials. 23
D. Additional studies not included in the original proposal 24
Study 11: The effect of product dimensions and surface browning method on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products treated with various antimicrobials. 24
Study 12: The effect of lauric arginate, and its combinations with caprylic acid, carvacrol, and/or ε-polylysine, on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products..... 25
VIII. CONCLUSIONS..... 26
A. Study addressing Objective 1..... 26
B. Studies addressing Objectives 2 and 3..... 26

C. STUDY ADDRESSING OBJECTIVE 4 27
D. ADDITIONAL STUDIES NOT INCLUDED IN THE ORIGINAL PROPOSAL 27
IX. RECOMMENDATIONS FOR FUTURE RESEARCH 27
X. PRESENTATIONS AND PUBLICATIONS 28
XI. REFERENCES 28
XII. FIGURES 30
XIII. APPENDIX 40

I. EXECUTIVE SUMMARY

Project Title: Development of an Intervention to Reduce the Likelihood of *Salmonella* Contamination in Raw Poultry Intended for use in the Manufacture of Frozen, Not-Ready-to-Eat Entrees

Principal Investigator: John N. Sofos
Co-Investigators: Galatios D. Moschonas, Ifigenia Geornaras, Jarret D. Stopforth, Damien Wach, Dale R. Woerner, Keith E. Belk, and Gary C. Smith

Research Institution: Center for Meat Safety & Quality, Department of Animal Sciences, Colorado State University, 1171 Campus Delivery, Fort Collins, CO 80523-1171

Submittal Date of Final Report to AMI: April 2011

Frozen, not-ready-to-eat breaded chicken products have been linked to salmonellosis outbreaks. The manufacturing process of these products involves a mild heating/browning step to maintain the shape of the product and provide it with a desirable golden-brown color prior to freezing and packaging; however, this step is not a complete lethality step and is not intended to fully-cook the product. Therefore, the risk associated with these products is that they may appear ready-to-eat and consequently be consumed uncooked or undercooked. The overall goal of the project was to identify antimicrobial ingredients for reducing levels of *Salmonella* contamination in frozen, not-ready-to-eat breaded chicken products. A study was initially conducted to screen four levels of 11 individual antimicrobial ingredients against *Salmonella* in raw chicken breast portions. Based on the findings of this study, four antimicrobials were selected for further evaluation as single or combination treatments, in a process simulating the manufacture of a frozen, not-ready-to-eat breaded chicken product. Additional studies evaluated the combined effect of different surface browning methods (i.e., oven baking or flash frying) and product dimensions [i.e., small (9 × 2.5 × 2 cm; 50 g) or large (9 × 5 × 3 cm; 150 g)] on *Salmonella* reductions in products formulated with selected antimicrobial treatments. Selected findings indicated that single treatments of caprylic acid (0.25 to 1.0%), carvacrol (0.3 to 0.5%), peracetic acid (0.3 and 0.5%), and ε-polylysine (0.5 and 1.0%) reduced *Salmonella* contamination in frozen, not-ready-to-eat breaded chicken products by 1.6 to at least 4.7 log CFU/g; combinations comprised of caprylic acid (0.0625 to 0.25%) + carvacrol (0.075 to 0.3%), caprylic acid (0.0625 to 0.25%) + ε-polylysine (0.5%) or carvacrol (0.075 to 0.3%) + ε-polylysine (0.5%) reduced *Salmonella* counts by 1.7 to at least 4.5 log CFU/g, depending on the treatment; and combinations comprised of all three ingredients (i.e., caprylic acid + carvacrol + ε-polylysine) reduced *Salmonella* counts by 2.4 to at least 4.6 log CFU/g, depending on the concentrations tested. Irrespective of antimicrobial treatment, oven browning of 9 × 2.5 × 2 cm (50 g) samples resulted in higher reductions of *Salmonella* than oven browning of 9 × 5 × 3 cm (150 g) samples; product dimensions did not affect pathogen reductions in samples surface-browned by flash frying. Fully cooked, uninoculated, breaded chicken products formulated with 0.5% ε-polylysine, alone, were found to be organoleptically acceptable by a sensory panel, compared to an untreated control (no antimicrobial ingredients), while products formulated with caprylic acid and/or carvacrol were less desirable. The findings of these studies may be useful for the selection of suitable antimicrobials, proper concentrations, and product manufacturing methods for reduction of *Salmonella* contamination in frozen, not-ready-to-eat, surface-browned, breaded chicken entrees.

II. TECHNICAL ABSTRACT

Commercial surface-browned, uncooked, frozen breaded chicken meat products may appear ready-to-eat, but in fact are raw; such products have been identified as sources of salmonellosis outbreaks. The overall goal of this project was to identify antimicrobial interventions for reduction of *Salmonella* contamination in such products. Specifically, studies (i) screened four levels of 11 individual ingredients for reducing *Salmonella* in raw chicken breast portions (Objective 1), (ii) evaluated the effect of individual ingredients and combinations thereof (from Objective 1) against *Salmonella* on raw ground chicken breast to be used in the preparation of a frozen, not-ready-to-eat breaded chicken product (Objective 2), (iii) evaluated the effect of the above ingredients (from Objective 2) on the resistance of *Salmonella* in a product that is subjected to undercooking and subsequent freezing (Objective 3), and (iv) evaluated the organoleptic properties of products treated with selected ingredients (Objective 4). Under Objective 1, raw chicken breast portions inoculated with *Salmonella* (6 log CFU/g) were treated with four levels of each of 11 antimicrobials, including allyl isothiocyanate (0.5 to 2.0%), caprylic acid (0.1 to 1.0%), carvacrol (0.1 to 0.5%), citric acid (0.5 to 2.0%), grapefruit distilled terpene (0.25 to 2.0%), malic acid (0.25 to 2.0%), oregano oil (0.1 to 0.5%), peracetic acid (0.1 to 0.5%), ε-polylysine (0.025 to 0.25%), sodium citrate (0.5 to 2.0%), and sodium lactate (0.5 to 2.0%). *Salmonella* counts were reduced by at least 4.5 log CFU/g compared to an untreated control in samples treated with 2.0% allyl isothiocyanate, 1.0% caprylic acid, 0.3 or 0.5% carvacrol, 0.5% oregano oil, or 0.5% peracetic acid. Furthermore, pathogen counts were reduced by 2.5 log CFU/g in samples treated with 0.25% ε-polylysine. Based on these findings, four antimicrobials (i.e., caprylic acid, carvacrol, peracetic acid, and ε-polylysine) were selected for further evaluation (under Objectives 2 and 3), as single or combination treatments, in a process simulating manufacture of a frozen, not-ready-to-eat breaded chicken product. Under Objectives 2 and 3, fresh chicken breast meat portions (5 × 5 × 5 cm) inoculated with *Salmonella* (7-strain mixture; 5 log CFU/g) were mixed with distilled water (control) or with each of seven levels of caprylic acid (CAA; 0.03125 to 1.0%), six levels of carvacrol (CAR; 0.0375 to 0.5%), two levels of peracetic acid (PAA; 0.3 and 0.5%), or four levels of ε-polylysine (POL; 0.125 to 1.0%), applied individually or in combinations of two or three, for a total of 46 treatments including controls. Sodium chloride (1.2%) and sodium tripolyphosphate (0.3%) were added to all treatments (5% total moisture enhancement level), and then ground and formed into 9 × 5 × 3 cm (150 g) portions. Samples were glazed with beaten egg whites, rolled in breadcrumbs, browned by oven-baking (208°C, 15 min), packaged in polyethylene bags, and stored at -20°C for 7 days. Total reductions of inoculated *Salmonella* in control samples ranged from 0.8 to 1.4 log CFU/g after frozen storage of the surface-browned breaded chicken product. In comparison, single treatments of CAA (0.25 to 1.0%), CAR (0.3 to 0.5%), PAA (0.3 and 0.5%) or POL (0.125 to 1.0%) reduced counts by 2.9 to at least 4.5, 3.4 to at least 4.6, 2.1 to at least 3.9, and 1.4 to 2.3 log CFU/g, respectively, depending on the tested concentration. Addition of PAA gave a product discoloration and an offensive odor, and was not examined further. For the rest of the antimicrobials, levels of 0.0625 to 0.25% CAA, 0.075 to 0.3% CAR or 0.5% POL, applied in combinations of two or three, reduced ($P<0.05$) *Salmonella* counts in stored frozen products by 1.7 to at least 4.6 log CFU/g. Specifically, combinations of CAA (0.0625 to 0.25%) + CAR (0.075 to 0.3%) reduced *Salmonella* counts by 1.8 to at least 4.3 log CFU/g. Combinations of 0.0625, 0.125 or 0.25% CAA with 0.5% POL reduced counts by 1.8, 1.7, and 2.6 log CFU/g, respectively, while combinations of 0.075, 0.15 or 0.3% CAR with 0.5% POL reduced counts by 2.0, 3.2, and at least 4.5 log CFU/g, respectively. Combinations comprised of all three

antimicrobials reduced ($P<0.05$) *Salmonella* counts by 2.4 to at least 4.6 log CFU/g, depending on the concentrations tested. Based on findings of the studies conducted under Objectives 2 and 3, antimicrobial interventions comprised of individual and combination treatments of CAA, CAR and POL that significantly ($P<0.05$) reduced *Salmonella* contamination in the frozen breaded chicken product, were selected for sensory evaluation (Objective 4). These treatments included CAA (0.25%), CAR (0.3%), POL (0.5%), CAA (0.06%) + CAR (0.08%), CAA (0.13%) + CAR (0.15%), CAA (0.25%) + POL (0.5%), CAR (0.15%) + POL (0.5%), CAR (0.3%) + POL (0.5%), CAA (0.06%) + CAR (0.08%) + POL (0.5%), and CAA (0.13%) + CAR (0.15%) + POL (0.5%). Fully cooked, uninoculated, breaded chicken products formulated with these antimicrobial treatments, as well as appropriate controls (i.e., no antimicrobial ingredients, and single treatments of CAA [0.06 or 0.13%] and CAR (0.08 or 0.15%]), were evaluated by a trained panel comprised of 5 to 6 participants. Based on this limited, exploratory sensory analysis, products formulated with 0.5% POL, alone, were found to be organoleptically acceptable by the panel compared to the untreated control (no antimicrobial ingredients), while products formulated with CAA and/or CAR were less desirable. More specifically, products formulated with CAA were described as having a "goat cheese taste" while products containing CAR were characterized as being "spicy" or having a "strong herbal flavor". Two additional microbiological studies, not included in the proposal, were conducted. The objective of one of these studies was to evaluate the effect of product dimensions and surface browning methods on reduction of *Salmonella* contamination in frozen, not-ready-to-eat breaded products formulated with selected antimicrobial ingredients. Selected findings of this study were that, irrespective of antimicrobial treatment, oven browning (208°C, 15 min) of 9 × 2.5 × 2 cm (50 g) samples resulted in higher ($P<0.05$) total reductions (reductions of at least 4.6 log CFU/g) of *Salmonella* than oven browning of 9 × 5 × 3 cm (150 g) samples (reductions of 1.6 to 2.5 log CFU/g); and, product dimensions did not ($P\geq0.05$) affect pathogen reductions in samples surface-browned by flash frying (190°C, 15 s) (reductions of 1.5 to 2.5 log CFU/g). The objective of the second study was to evaluate the effect of 0.02% lauric arginate (LAE) in combinations with 0.5% POL and/or 0.03% CAA + 0.04% CAR against *Salmonella* in frozen, breaded, browned (by fryer or oven browning) chicken products. Results showed that LAE was not ($P\geq0.05$) effective against *Salmonella* when tested on its own, and it did not ($P\geq0.05$) enhance the antimicrobial activity of 0.5% POL and/or 0.03% CAA + 0.04% CAR. Overall, the data presented in this report may be useful for the selection of suitable antimicrobials, proper concentrations, product sizes, and product surface browning methods for reducing levels of *Salmonella* contamination in frozen, not-ready-to-eat, surface-browned, breaded chicken entrees.

III. INTRODUCTION

Salmonella is a frequent cause of foodborne illness worldwide, with estimates in the United States alone suggesting that this pathogen causes 1 million cases of illness, 20,000 hospitalizations, and more than 350 deaths annually (Scallan et al., 2011). Frozen, breaded chicken products containing raw poultry that appear ready-to-eat but in fact are partly cooked or browned, specifically raw, frozen chicken nuggets, strips, and other entrees, have been identified as sources of salmonellosis outbreaks in Australia (Kenny et al., 1999), British Columbia (Currie et al., 2008; MacDougall et al., 2004), and the United States (Smith et al., 2008). In the United States alone, between 1998 and 2008, six separate outbreaks occurred in Minnesota (additional states were involved in some of these outbreaks) involving raw, frozen, microwaveable, breaded, pre-browned and stuffed chicken products (Smith et al., 2008).

Manufacture of raw, frozen, breaded chicken products involves use of raw chicken meat that undergoes particle size reduction to improve protein extraction and binding of meat pieces with the subsequent addition of binding ingredients, such as salt and phosphates. Once product is formed, it undergoes a partial cooking/browning (fried or baked) step to maintain the shape of the product and induce a desirable golden-brown color prior to freezing and packaging; however, this step is not a complete lethality step and is not intended to fully-cook the product.

Since the chicken meat used is raw and is comminuted during manufacturing, the bacteriological quality of these products should be considered the same as raw poultry. The risk with these products is that they do not appear raw, and may lead consumers to treat them with less precaution than they typically would a visibly raw product. Investigations by the Minnesota Department of Health and the Minnesota Department of Agriculture (Smith et al., 2008) indicated that in most of these cases, consumers did not follow the manufacturer cooking instructions on the package label. It was also shown that microwave cooking was one of the primary factors contributing to these outbreaks.

A guidance document (USDA/FSIS, 2006) was prepared by the U.S. Department of Agriculture Food Safety and Inspection Service entitled "Labeling Policy Guidance: Uncooked, Breaded, Boneless Poultry Products" for processors to ensure sufficient information was provided to the consumer on the package regarding safe handling instructions (especially regarding adequate cooking). Yet, there is still concern that consumers may undercook these products, thus making them a significant risk factor in contracting foodborne salmonellosis. Therefore, there is a clear need for the industry to take additional measures for reducing the risk of *Salmonella* survival in these types of products, and a sentiment that has emerged from the attention to these types of products is a need for intervention(s) that reduce the likelihood of contamination. In this report, we describe the steps that were taken to assess the antimicrobial effect of several compounds on *Salmonella* in breaded chicken entrees, and present the data that have been collected, as described in the project objectives below.

IV. OVERALL PROJECT GOAL

The overall goal of this research was to identify antimicrobial ingredients for reducing levels of *Salmonella* contamination in raw ground chicken used in the preparation of frozen, not-ready-to-eat breaded chicken products.

V. SUPPORTING OBJECTIVES

The overall project goal was supported by the following objectives:

1. Screen four levels of 11 individual ingredients for reducing *Salmonella* in raw chicken breast.

2. Evaluate the effect of 30 treatments composed of individual ingredients and combinations thereof (from Objective 1) against *Salmonella* on raw ground chicken breast to be used in the preparation of frozen, not-ready-to-eat breaded chicken products.

3. Evaluate the effect of the 30 treatments (from Objective 2) on the resistance of *Salmonella* in product that is subjected to undercooking and subsequent freezing.

4. Evaluate the organoleptic properties of products treated with the interventions.

Work conducted under Objectives 2 and 3 was done by Colorado State University, and work conducted under Objectives 1 and 4 was funded and done by PURAC.

VI. MATERIALS AND METHODS

A. Study addressing Objective 1

<u>Study 1</u>: Evaluation of four levels of 11 individual ingredients against *Salmonella* in diced, raw chicken breast.

As indicated, this work was funded and performed by PURAC.

***Salmonella* strains and inoculum preparation.** The inoculum was comprised of *Salmonella* Enteritidis ATCC 13076 and *Salmonella* Typhimurium ATCC 13311. The strains were individually cultured twice to stationary phase of growth in brain heart infusion broth (Oxoid Ltd., Basingstoke, Hampshire, UK) for 18-24 h at 37°C. The cultures were then combined and a 5 ml aliquot of the mixture was diluted in 100 ml of diluent comprised of 0.85% (w/w) NaCl (Merck KGaA, Darmstadt, Germany) and 0.1% (w/w) bacteriological peptone (Oxoid Ltd.).

Inoculation and treatment of raw chicken breast meat. Frozen, diced chicken breast meat was thawed at 4°C overnight, and then divided into 100 g batches and inoculated with 1 ml of the diluted *Salmonella* inoculum. The target inoculation level was approximately 6 log CFU/g. The meat was held at 4°C for 30 min to allow for bacterial cell attachment and then mixed for 2 min with one of the following antimicrobial treatments; as indicated, four concentrations of each antimicrobial were tested:

1. No treatment (control)
2. Allyl isothiocyanate (Acros Organics, Geel, Belgium) (0.5, 1.0, 1.5, and 2.0%, v/w)
3. Caprylic acid (Fisher Scientific, Hampton, NH) (0.1, 0.25, 0.5, and 1.0%, v/w)
4. Carvacrol (Acros Organics) (0.1, 0.2, 0.3, and 0.5%, v/w)
5. Citric acid (Acros Organics) (0.5, 1.0, 1.5, and 2.0%, v/w)
6. Grapefruit distilled terpene (Moellhaussen, Milano, Italy) (0.25, 0.5, 1.0, and 2.0%, v/w)
7. Malic acid (Merck, Darmstadt, Germany) (0.25, 0.5, 1.0, and 2.0%, v/w)
8. Oregano oil (Aldrich, St. Louis, MO) (0.1, 0.2, 0.3, and 0.5%, v/w)
9. Peracetic acid (Acros Organics) (0.1, 0.2, 0.3, and 0.5%, v/w)
10. ε-Polylysine (Chisso Corporation, Minamata, Japan) (0.025, 0.0625, 0.125, and 0.25%, v/w; i.e., 0.1, 0.25, 0.5, and 1.0%, respectively, of the 25% commercially available ε-polylysine solution)
11. Sodium citrate (Acros Organics) (0.5, 1.0, 1.5, and 2.0%, v/w)
12. Sodium lactate (Purasal S, PURAC, Gorinchem, The Netherlands) (0.5, 1.0, 1.5, and 2.0%, v/w).

After treatment, chicken meat samples were held for 8 min (to simulate the process whereby such a treatment may be added in a commercial setting involving mixing and portioning of raw poultry portions for further processing into frozen, not-ready-to-eat breaded chicken products) before microbiological analysis.

Microbiological analysis. For enumeration of surviving bacterial populations, untreated (control) or treated samples were placed in a stomacher bag to which two times the net weight of diluent was added. The samples were homogenized, serially diluted and plated on tryptic soy agar (TSA, Oxoid) and violet red bile glucose (VRBG) agar (Oxoid) using an Eddyjet type 1.23-spiral plater (IUL Instruments, Barcelona, Spain). Colonies were enumerated after incubation of plates at 37°C for 24 h. Two replicate trials were conducted, with three samples analyzed per antimicrobial treatment in each replicate.

B. Studies addressing Objectives 2 and 3

Work addressing Objectives 2 and 3 of the project was conducted concurrently. As previously stated, this work was performed by Colorado State University.

Based on the findings of Study 1, four antimicrobials were selected for further evaluation in a process simulating manufacture of a frozen, not-ready-to-eat breaded chicken product. The four antimicrobials selected were carvacrol (the most effective essential oil), caprylic acid and peracetic acid (the two most effective acids), and ε-polylysine (a cationic surfactant that reduced *Salmonella* levels by 2.5 log CFU/g at a concentration of 0.25%). Grapefruit terpenes and the organic acid salts (i.e., sodium lactate and sodium citrate) were not selected for further testing since results from the screening study (Study 1) indicated that they had limited or no effect against *Salmonella*. Allyl isothiocyanate was also not selected because it showed efficacy only at the higher tested concentrations (i.e., 1.5 and 2.0%); use of allyl isothiocyanate at these levels would result in undesirable effects on product taste and odor.

Several studies were conducted to evaluate the antimicrobial effects of different concentrations of caprylic acid, carvacrol, peracetic acid, and ε-polylysine, applied individually or in combinations of two or three, against *Salmonella* during production and frozen storage of a not-ready-to-eat breaded chicken product. This work is described under Studies 2 to 9.

Prior to initiation of these studies, various commercial frozen, not-ready-to-eat breaded chicken products were purchased from local supermarkets to examine their size and dimensions. The most common dimensions of the products were 9 cm length × 5 cm width × 3 cm height; thus, these dimensions were used for the product prepared in our studies. Furthermore, based on the manufacturer's ingredients statement on the packaging label of the commercial products, the only method used for surface browning of these products was flash frying in vegetable oil. Therefore, in addition to what was stated in the project proposal, browning of products of selected antimicrobial treatments was done using both the oven-baking method (208°C for 15 min), as stated in the proposal, and a deep-frying method (190°C for 15 s).

<u>Study 2</u>: The effect of various concentrations of caprylic acid, carvacrol, peracetic acid, and ε-polylysine on reductions of *Salmonella* contamination in frozen, oven-browned, breaded chicken products.

A flow chart illustrating the procedure that was followed for inoculation, treatment, and preparation of the frozen, surface-browned, not-ready-to-eat breaded chicken products is shown in Figure 1.

***Salmonella* strains and inoculum preparation.** The inoculum was comprised of seven *Salmonella* strains (kindly provided by Dr. Vijay Juneja, Microbial Food Safety Research Unit, ERRC-ARS-USDA, Wyndmoor, PA) isolated from chicken or turkey:

- *Salmonella* Kentucky FSIS 062/VJS1 (chicken)
- *Salmonella* Kentucky FSIS 044/VJS2 (chicken)
- *Salmonella* Hadar FSIS 064/VJS6 (chicken)
- *Salmonella* Thompson FSIS 132/VJS7 (chicken)
- *Salmonella* Muenster FSIS MF61976/VJS15 (turkey)
- *Salmonella* Reading FSIS MF58210/VJS17 (turkey)
- *Salmonella* Hadar FSIS MF61777/VJS19 (turkey)

All seven strains formed colonies with black centers on xylose lysine deoxycholate (XLD) agar (Acumedia, Lansing, MI) indicating hydrogen sulfide production. The strains were individually activated and subcultured in 10 ml tryptic soy broth (Difco, Becton Dickinson, Sparks, MD) for 18-24 h at 35°C. The cultures were then combined, harvested by centrifugation (4,629×*g*, 15 min, 4°C; Eppendorf model 5810 R, Brinkmann Instruments Inc., Westbury, NY) and washed twice in 10 ml phosphate-buffered saline (PBS, pH 7.4; 0.2 g/L KH_2PO_4, 1.5 g/L $Na_2HPO_4 \cdot 7H_2O$, 8.0 g/L NaCl and 0.2 g/L KCl). The washed cell pellet was resuspended in 70 ml PBS and further diluted in PBS to a concentration of 6 to 7 log CFU/ml.

Inoculation, treatment, product preparation, and frozen storage. Fresh, boneless, skinless chicken breasts (with no added chicken broth) were purchased directly from a poultry processing facility in Colorado. If not used within 24 h, the chicken breasts were vacuum-packaged and stored at -20°C. When needed, they were thawed at 4°C for approximately 48 h before use. The chicken breast meat was cut into pieces (approximately 5 × 5 × 5 cm), and batches of 2 kg were inoculated with 20 ml of the *Salmonella* inoculum to a target level of 4-5 log CFU/g. The chicken meat and inoculum were thoroughly mixed for 2 min using a KitchenAid Professional 600™ mixer (KitchenAid, St. Joseph, MI) at a speed setting of "stir", and then left to stand at 4°C for 30 min for bacterial cell attachment. The inoculated batches of chicken meat were then treated with one of the following treatments; as indicated, two levels of each antimicrobial were tested:

1. Sterile distilled water (control)
2. Caprylic acid (CAA; 0.5 and 1.0%, v/w)
3. Carvacrol (CAR; 0.3 and 0.5%, v/w)
4. Peracetic acid (Sigma-Aldrich, St. Louis, MO) (PAA; 0.3 and 0.5%, v/w)
5. ε-Polylysine (POL; 0.125 and 0.25%, v/w; i.e., 0.5 and 1.0%, respectively, of the 25% ε-polylysine solution)

The inoculated chicken portions were mixed with the distilled water or antimicrobial solution for 5 min, followed by addition and mixing (5 min) of sodium chloride (Fisher Scientific) and sodium tripolyphosphate (kindly provided by BK Giulini Corporation, Simi Valley, CA) to yield concentrations of 1.2 and 0.3% (w/w), respectively, in the final product. The total moisture enhancement level of the final product was 5%. The mixture was then ground (0.6 cm grinder plate) with a TSM#8 electric meat grinder (The Sausage Maker Inc., Buffalo, NY), and formed into rectangular (9 cm length × 5 cm width × 3 cm height) 150 g portions. The portions were then glazed with beaten pasteurized egg whites (All Whites, Crystal Farm, Lake Mills, WI) and rolled in plain breadcrumbs (Kroger, Cincinnati, OH), followed by browning for 15 min in a standard kitchen oven (Magic Chef, Maytag Corp., Newton, IA) set at 208°C. The temperature of the oven chamber and the geometric center of products was monitored and recorded at 120 s intervals during browning, using type-K thermocouples and PicoLog data acquisition software (Pico Technology, Ltd., Cambridge, UK). Samples were flipped over halfway (7.5 min) during the browning period. After browning, samples were analyzed for surviving bacterial populations within 2 to 3 min after they were removed from the oven. Additional samples were allowed to cool and were then individually packaged in double zipper bags (Ziploc, S.C. Johnson, Racine, WI) and stored at -20°C for 7 days.

Microbiological and physicochemical analyses. As shown in Figure 1, samples were analyzed for microbial counts at four points of the process: (1) after inoculation, (2) after grinding (i.e., approximately 15 min after antimicrobial addition), (3) after browning, and (4) after 7 days of

frozen (-20°C) storage. For sampling points (1) and (2), 25 g samples were analyzed, whereas for sampling points (3) and (4), samples were comprised of the entire 150 g breaded chicken product. Frozen samples (sampling point 4) were thawed for 15-18 h at 4°C before microbial analysis. Samples (25 or 150 g) were placed in a Whirl-Pak filter bag (Nasco, Modesto, CA), to which maximum recovery diluent (MRD; 0.85% NaCl and 0.1% peptone) was added at a 1:1 ratio of sample weight (g) / volume (ml) of MRD. The samples were homogenized (Masticator, IUL Instruments) for 2 min, serially diluted in 0.1% buffered peptone water (Difco, Becton Dickinson), and surface-plated for *Salmonella* counts on XLD agar, and total bacterial counts on tryptic soy agar (Acumedia) supplemented with 0.1% sodium pyruvate (Fisher Scientific, Pittsburgh, PA) (TSAP). Colonies were enumerated after incubation of plates at 35°C for 24 h (XLD agar) or 25±2°C for 72 h (TSAP). The detection limit of the analysis was 0.3 log CFU/g. Uninoculated chicken breast meat samples were also analyzed to determine the natural microbial contamination level.

After microbial analysis, pH measurements were taken of the sample homogenates with a Denver Instruments (Arvada, CO) pH meter fitted with a glass electrode. Water activity values (AquaLab model series 3, Decagon Devices, Pullman,WA) of the surface-browned, breaded chicken products were also determined.

Statistical analysis. At each sampling point, three samples per treatment were analyzed in each of two repetitions of the experiment. The pH, water activity, and microbiological (converted to log CFU/g) data were analyzed with the PROC MIXED procedures of SAS (version 9.2, SAS Institute Inc., Cary, NC) with independent variables including antimicrobial treatment, sampling point, and their interaction. Means were separated with the least significant difference procedure at the significance level of α=0.05.

<u>Study 3</u>: The effect of various concentrations of caprylic acid, carvacrol, peracetic acid, and ε-polylysine on reductions of *Salmonella* contamination in frozen, fryer-browned, breaded chicken products.

In this study, the same methodology and antimicrobial treatments described in Study 2 was repeated, but this time, the treated, breaded samples were browned by deep-frying (190°C, 15 s) in 3 liters of vegetable oil (Pure Wesson Vegetable Oil, ConAgra Foods, Omaha, NE), using a Presto Digital Pro Fry deep fryer (Eau Claire, WI). The temperature of the vegetable oil in the deep fryer and the geometric center of products was continuously monitored and recorded at 1 s intervals during browning, as described in Study 2.

Two separate replicates of the study were conducted, with three samples analyzed per replicate at each sampling point. Data collected (i.e., *Salmonella* counts, total bacterial counts, pH, and water activity values) were statistically analyzed as described in Study 2.

<u>Study 4</u>: The effect of various concentrations of caprylic acid on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

Based on findings of Study 2, additional studies were carried out to test additional concentrations of caprylic acid (Study 4), carvacrol (Study 5), and ε-polylysine (Study 6), applied individually. Use of peracetic acid was excluded from this and future experiments since it was found to result in product discoloration and an offensive odor, thus making this ingredient unsuitable for use in this type of application, at least under the conditions described in this report, and the concentrations evaluated (0.3 and 0.5%).

In Study 4, various concentrations of caprylic acid were evaluated for their effect against *Salmonella* in the frozen, not-ready-to-eat breaded chicken product. The procedure described in Study 2 was followed to prepare inoculated products formulated with 0.25, 0.5, 0.75, and 1.0% (v/w) caprylic acid; sterile distilled water was used as the control treatment. Two separate replicates of the study were conducted, with three samples analyzed per replicate at each sampling point. Data collected (i.e., *Salmonella* counts, total bacterial counts, pH, and water activity values) were statistically analyzed as described in Study 2.

Study 5: The effect of various concentrations of carvacrol on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

The methodology described in Study 2 was used to evaluate the effect of various concentrations of carvacrol against *Salmonella* in the frozen, not-ready-to-eat breaded chicken product. The concentrations of carvacrol tested were 0.3, 0.4, and 0.5% (v/w), and sterile distilled water was used as the control. Two separate replicates of the study were conducted, with three samples analyzed per replicate at each sampling point. Data collected (i.e., *Salmonella* counts, total bacterial counts, pH, and water activity values) were statistically analyzed as in Study 2.

Study 6: The effect of various concentrations of ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

In this study, four concentrations of ε-polylysine were evaluated for their effect against *Salmonella* in the frozen, not-ready-to-eat breaded chicken product. The methodology described in Study 2 was used to prepare inoculated products formulated with 0.125, 0.25, 0.5, and 1.0% (v/w) ε-polylysine (i.e., 0.5, 1.0, 2.0, and 4.0% of the 25% ε-polylysine solution). As before, sterile distilled water was used as the control treatment. Two separate replicates of the study were conducted, with three samples analyzed per replicate at each sampling point. Data collected (i.e., *Salmonella* counts, total bacterial counts, pH, and water activity values) were statistically analyzed as described in Study 2.

Study 7: The effect of combinations of 0.25% caprylic acid, 0.3% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

In Studies 7 to 9, caprylic acid, carvacrol, and ε-polylysine were tested at different concentrations, in combinations of two or three, for their effect against *Salmonella* in the frozen, not-ready-to-eat breaded chicken product.

In Study 7, various combinations of 0.25% caprylic acid, 0.3% carvacrol, and 0.5% ε-polylysine were evaluated using the methodology described in Study 2. The treatments tested were:

1. Sterile distilled water (control)
2. CAA (0.25%)
3. CAR (0.3%)
4. POL (0.5%)
5. CAA (0.25%) + CAR (0.3%)
6. CAA (0.25%) + POL (0.5%)
7. CAR (0.3%) + POL (0.5%)
8. CAA (0.25%) + CAR (0.3%) + POL (0.5%)

Two separate replicates of the study were conducted, with three samples analyzed per replicate at each sampling point. Data collected (i.e., *Salmonella* counts, total bacterial counts, pH, and water activity values) were statistically analyzed as described in Study 2.

<u>Study 8</u>: The effect of combinations of 0.125% caprylic acid, 0.15% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

Based on *Salmonella* reductions obtained for treatments evaluated under Study 7, it was decided to evaluate lower levels of caprylic acid and carvacrol; thus, the previously tested concentrations (0.25% and 0.3%, respectively) of these ingredients in combination treatments were reduced by half. As before, the procedure described in Study 2 was used to prepare inoculated products formulated with various combinations of 0.125% caprylic acid, 0.15% carvacrol, and 0.5% ε-polylysine. Some of the antimicrobial treatments evaluated in Study 7 were repeated here as control treatments. The treatments tested were:

1. Sterile distilled water
2. CAA (0.125%) + CAR (0.15%)
3. CAA (0.25%) + CAR (0.3%)
4. CAA (0.125%) + POL (0.5%)
5. CAA (0.25%) + POL (0.5%)
6. CAR (0.15%) + POL (0.5%)
7. CAR (0.3%) + POL (0.5%)
8. CAA (0.125%) + CAR (0.15%) + POL (0.5%)
9. CAA (0.25%) + CAR (0.3%) + POL 0.5%)

Two separate replicates of the study were conducted, with three samples analyzed per replicate at each sampling point. Data collected (i.e., *Salmonella* counts, total bacterial counts, pH, and water activity values) were statistically analyzed as described in Study 2.

<u>Study 9</u>: The effect of combinations of 0.03125% or 0.0625% caprylic acid, 0.0375% or 0.075% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

In this study, previously (Study 8) tested concentrations of caprylic acid and carvacrol (i.e., 0.125% and 0.15%, respectively) were again reduced and tested at levels of 0.03125 or 0.0625% caprylic acid and 0.0375 or 0.075% carvacrol in combinations with 0.5% ε-polylysine. The methodology described in Study 2 was used to evaluate the following treatments for their effect against *Salmonella* in the frozen, not-ready-to-eat breaded chicken product:

1. Sterile distilled water (control)
2. CAA (0.0625%) + CAR (0.075%)
3. CAA (0.0625%) + POL (0.5%)
4. CAR (0.075%) + POL (0.5%)
5. CAA (0.03125%) + CAR (0.0375%) + POL (0.5%)
6. CAA (0.0625%) + CAR (0.075%) + POL (0.5%)

Two separate replicates of the study were conducted, with three samples analyzed per replicate at each sampling point. Data collected (i.e., *Salmonella* counts, total bacterial counts, pH, and water activity values) were statistically analyzed as described in Study 2.

C. Study addressing Objective 4

<u>Study 10</u>: Evaluation of the organoleptic properties of fully cooked, breaded chicken products treated with selected antimicrobials.

As previously stated, this work was funded and performed by PURAC.

The overall purpose of this work was to obtain preliminary data on the organoleptic properties of individual and combination antimicrobial treatments identified from microbiological data, obtained under Objectives 2 and 3 (Studies 2 to 9), to significantly ($P<0.05$) reduce *Salmonella* contamination in the frozen, not-ready-to-eat breaded chicken product. The antimicrobial treatments selected for sensory analysis included:

1. CAA (0.25%)
2. CAR (0.3%)
3. POL (0.5%)
4. CAA (0.06%) + CAR (0.08%)
5. CAA (0.13%) + CAR (0.15%)
6. CAA (0.25%) + POL (0.5%)
7. CAR (0.15%) + POL (0.5%)
8. CAR (0.3%) + POL (0.5%)
9. CAA (0.06%) + CAR (0.08%) + POL (0.5%)
10. CAA (0.13%) + CAR (0.15%) + POL (0.5%)

Given that caprylic acid and carvacrol are known to have strong sensory characteristics, additional concentrations of these antimicrobials were included in the sensory analysis to serve as controls for the combination treatments; these control treatments were:

1. CAA (0.06%)
2. CAA (0.13%)
3. CAR (0.08%)
4. CAR (0.15%)

Uninoculated, breaded chicken products formulated with the above antimicrobial treatments, as well as an untreated control (no antimicrobial ingredients) were prepared on the same day, as outlined in Figure 2. The products were frozen after preparation, and were thawed and fully cooked when needed. The sensory evaluation was conducted with a trained panel of 5 to 6 participants, and was conducted in four parts:

- For Part 1, the following four treatments were assessed by 5 individuals:
  - → Control (no antimicrobials)
  - → CAA (0.06%)
  - → CAA (0.13%)
  - → CAA (0.25%)
- For Part 2, the following four treatments were assessed by 6 individuals:
  - → Control (no antimicrobials)
  - → CAR (0.08%)
  - → CAR (0.15%)
  - → CAR (0.3%)
- For Part 3, the following five treatments were assessed by 5 individuals:
  - → Control (no antimicrobials)
  - → POL (0.5%)

→ CAA (0.06%) + CAR (0.08%)
→ CAA (0.13%) + CAR (0.15%)
→ CAA (0.25%) + POL (0.5%)

- For Part 4, the following five treatments were assessed by 6 individuals:
  → Control (no antimicrobials)
  → CAR (0.15%) + POL (0.5%)
  → CAR (0.3%) + POL (0.5%)
  → CAA (0.06%) + CAR (0.08%) + POL (0.5%)
  → CAA (0.13%) + CAR (0.15%) + POL (0.5%)

For Parts 1 and 2, a threshold taste test was used since caprylic acid and carvacrol were tested at different levels and they are known to have a strong flavor. The panelists tasted samples containing increasing concentrations of caprylic acid or carvacrol until they considered a sample unacceptable based on taste. For Parts 3 and 4, panelists were asked to judge the samples and rank them on acceptability when it was possible. In all cases, the following scale was used to assess the treatments for intensity of odor, taste, and mouth feel, as compared to the control (no antimicrobials added): 0 = same as control; 1 = very weakly different to the control; 2 = weakly different to the control; 3 = clearly different to the control; 4 = strongly different to the control; and 5 = very strongly different to the control.

D. Additional studies not included in the original proposal

Two additional microbiological studies, not included in the proposal, were conducted by Colorado State University.

<u>Study 11</u>: The effect of product dimensions and surface browning method on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products treated with various antimicrobials.

Studies 2 to 9 were all conducted on the same-sized product; therefore, it was decided to conduct a study to determine the effect of product size on reductions of *Salmonella* contamination during preparation of a frozen, not-ready-to-eat breaded chicken product. Furthermore, it was deemed necessary to directly compare the effect of the two surface browning methods (i.e., oven browning and flash frying) on pathogen reductions. Therefore, the objective of this study was to evaluate the effect of product dimensions and browning methods on reductions of *Salmonella* counts in frozen, not-ready-to-eat breaded samples treated with combinations of antimicrobials.

The procedure described in Study 2 was followed to inoculate and prepare the not-ready-to-eat breaded samples. The treatments tested are listed below (the antimicrobial treatments constitute the lowest tested concentrations of combination treatments selected for sensory evaluation under Study 10):

1. Sterile distilled water (control)
2. CAA (0.0625%) + CAR (0.075%)
3. CAA (0.25%) + POL (0.5%)
4. CAR (0.15%) + POL (0.5%)
5. CAA (0.0625%) + CAR (0.075%) + POL (0.5%)

Samples were formed into (i) 9 × 5 × 3 cm 150 g portions or (ii) 9 × 2.5 × 2 cm 50 g portions, and were browned in the (i) oven (208°C, 15 min) or (ii) deep fryer containing 3 liters of vegetable oil (190°C, 15 s), as previously described in Studies 2 and 3, respectively. Samples

were analyzed for microbial survivors and pH after inoculation, after surface browning, and after frozen storage (-20°C, 8 days), as described in Study 2. Two separate replicates of the study were conducted, with three samples analyzed per replicate at each sampling point. Data collected (i.e., *Salmonella* counts, total bacterial counts, and pH values) were statistically analyzed as described in Study 2.

Study 12: The effect of lauric arginate, and its combinations with caprylic acid, carvacrol, and/or ε-polylysine, on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

Based on findings of the sensory evaluation (Study 10), it was decided to evaluate 0.5% ε-polylysine (found to be organoleptically acceptable) in combination with lauric arginate (LAE), for possible enhancement of antimicrobial effects obtained with ε-polylysine alone. Furthermore, LAE was tested together with caprylic acid + carvacrol at concentrations lower than those evaluated in the sensory analysis.

The procedure described in Study 2 was followed to inoculate and prepare frozen, not-ready-to-eat breaded samples formulated with:

1. Sterile distilled water (control)
2. LAE (Protect-M, PURAC) (0.02%)
3. POL (0.5%)
4. LAE (0.02%) + POL (0.5%)
5. CAA (0.03%) + CAR (0.04%)
6. CAA (0.03%) + CAR (0.04%) + POL (0.5%)
7. CAA (0.03%) + CAR (0.04%) + POL (0.5%) + LAE (0.02%)

Samples were formed into 9 × 5 × 3 cm 150 g portions and were browned in the (i) oven (208°C, 15 min) or (ii) deep fryer (190°C, 15 s), as previously described in Studies 2 and 3, respectively. Samples were analyzed for microbial survivors and pH after inoculation, after surface browning, and after frozen storage (-20°C, 7 days), as described in Study 2. Two separate replicates of the study were conducted, with three samples analyzed per replicate at each sampling point. Data collected (i.e., *Salmonella* counts, total bacterial counts, and pH values) were statistically analyzed as described in Study 2.

VII. RESULTS

A. Study addressing Objective 1

Study 1: Evaluation of four levels of 11 individual ingredients against *Salmonella* in diced, raw chicken breast.

- Several antimicrobials were effective against *Salmonella* and reduced counts by at least 4.5 log CFU/g at the highest concentration tested; these antimicrobials included 2.0% allyl isothiocyanate, 1.0% caprylic acid, 0.3 or 0.5% carvacrol, 0.5% oregano oil, and 0.5% peracetic acid. Additionally, 0.25% ε-polylysine reduced pathogen counts by 2.5 log CFU/g.
- Pathogen reductions of <1 log CFU/g, or no reductions were obtained by all the other tested acids or acid salts (i.e., citric acid, malic acid, sodium lactate, and sodium citrate), and grapefruit terpenes.
- Based on the results of this study, four antimicrobials were selected for further testing under Objectives 2 and 3; specifically, carvacrol (the most effective essential oil),

caprylic acid and peracetic acid (the two most effective acids), and ε-polylysine (a cationic surfactant). As indicated under the Materials and Methods section, allyl isothiocyanate was not selected for further testing because it was only effective at concentrations (i.e., 1.5 and 2.0%) that would result in undesirable effects on product taste and odor.

B. Studies addressing Objectives 2 and 3

<u>Study 2</u>: The effect of various concentrations of caprylic acid, carvacrol, peracetic acid, and ε-polylysine on reductions of *Salmonella* contamination in frozen, oven-browned, breaded chicken products.

- *Salmonella* counts in inoculated (4.8±0.1 log CFU/g) control samples were reduced ($P<0.05$) by 1.2 log CFU/g after frozen storage (-20°C, 7 days) of oven-browned, breaded products. Oven browning alone (i.e., without subsequent frozen storage) reduced ($P\geq0.05$) initial pathogen contamination levels by 0.4 log CFU/g.
- For samples formulated with antimicrobials, both the antimicrobial treatment and the sampling point (as indicated in Figure 1) had a significant ($P<0.05$) effect on *Salmonella* and total bacterial counts. More specifically, compared to the control, all the tested antimicrobials and antimicrobial concentrations, except POL, significantly ($P<0.05$) reduced *Salmonella* counts in the raw ground chicken with salt and phosphate mixture (i.e., approximately 15 min after addition of the antimicrobial solution). At this sampling point, the most effective treatments, with at least 4.0 log CFU/g reductions in *Salmonella* counts compared to initial levels, were 1.0% CAA and 0.5% CAR.
- In most cases, additional reductions in *Salmonella* counts were obtained after oven browning of the treated breaded products and/or after 7 days of frozen storage. In particular, for samples treated with 1.0% CAA or 0.5% CAR, *Salmonella* counts were reduced to below the detection limit (<0.3 log CFU/g) after storage of products at -20°C for 7 days.
- Addition of 0.3 or 0.5% PAA to product formulations significantly ($P<0.05$) lowered *Salmonella* counts at all sampling points, compared to the control. However, even at the lowest tested concentration (i.e., 0.3%), it was noted that samples treated with PAA had an offensive odor and resulted in product discoloration. It was, thus, decided to discontinue testing of this antimicrobial after completion of Study 3.
- *Salmonella* counts obtained after browning or after frozen storage of samples treated with 0.125 or 0.25% POL were not ($P\geq0.05$) different than *Salmonella* counts obtained after browning or after frozen storage of the control samples.
- Overall, total pathogen reductions for samples treated with CAA (0.5 or 1.0%), CAR (0.3 or 0.5%), PAA (0.3 or 0.5%), or POL (0.125 or 0.25%) were 4.1 to >4.5, >4.0, 2.1 to >3.9, and 1.5 to 1.6 log CFU/g, respectively, after frozen storage of oven-browned samples. Compared to the control, all antimicrobials and antimicrobial concentrations tested, except POL (0.125 or 0.25%), significantly ($P<0.05$) reduced *Salmonella* counts in the final, frozen product.
- Uninoculated chicken breast samples analyzed for natural microbial contamination levels had total bacterial counts of 4.7±0.8 log CFU/g. Hydrogen sulfide-producing *Salmonella* populations were not detected (<0.3 log CFU/g) in any of the uninoculated samples.
- The pH of control samples after oven browning and frozen storage was 6.04. Compared to the control, formulation of products with 1.0% CAA or PAA (0.3 or 0.5%) lowered

(P<0.05) the pH of samples to 5.77, and 5.43 to 5.75, respectively, whereas formulation of products with POL (0.125 or 0.25%) increased (P<0.05) the pH to 6.18 to 6.20.
- Water activities of the control and samples formulated with CAA (0.5 or 1.0%), CAR (0.3 or 0.5%), PAA (0.3 or 0.5%), or POL (0.125 or 0.25%) were 0.978, 0.976 to 0.977, 0.978 to 0.980, 0.972 to 0.974, and 0.975 to 0.977, respectively, after oven browning.
- The average temperature of the geometric center of samples from all treatments reached a maximum of 44.6±3.5°C during the 15 min oven browning period (Figure 3).

<u>Study 3</u>: The effect of various concentrations of caprylic acid, carvacrol, peracetic acid, and ε-polylysine on reductions of *Salmonella* contamination in frozen, fryer-browned, breaded chicken products.

- Initial levels (4.9±0.2 log CFU/g) of *Salmonella* in inoculated control samples were reduced (P<0.05) by 0.8 log CFU/g in the final, frozen, fryer-browned product.
- The antimicrobial treatments evaluated in this study were identical to those of Study 2; therefore, as expected, *Salmonella* counts of samples analyzed approximately 15 min after addition of the antimicrobial solutions were similar to those obtained in Study 2. As in Study 2, compared to the control, all the tested antimicrobials and antimicrobial concentrations, except POL, significantly (P<0.05) reduced *Salmonella* counts in the raw ground chicken with salt and phosphate mixture (i.e., sampling point # 2; Figure 1).
- Irrespective of antimicrobial treatment, *Salmonella* counts of samples analyzed after fryer browning were not (P≥0.05) different than counts of samples analyzed approximately 15 min after addition of the antimicrobial.
- Compared to pathogen counts of samples analyzed after fryer browning, frozen storage of samples treated with 0.5% CAA, 0.3% CAR, 0.5% CAR, 0.5% PAA or 0.25% POL resulted in additional pathogens reductions (P<0.05) of 1.1, 1.4, >1.0, 0.7 and 0.6 log CFU/g, respectively.
- Overall, total *Salmonella* reductions for samples treated with CAA (0.5 or 1.0%), CAR (0.3 or 0.5%), PAA (0.3 or 0.5%), or POL (0.125 or 0.25%) were 3.3 to >4.3, 4.1 to >4.7, 1.6 to 2.5, and 1.1 log CFU/g, respectively, after frozen storage of fryer-browned samples. As seen in Study 2, compared to the control, all the tested antimicrobials and antimicrobial concentrations, except POL, significantly (P<0.05) reduced initial *Salmonella* counts in the final, frozen product.
- *Salmonella* (i.e., hydrogen sulfide-producing populations) and total bacterial counts of uninoculated chicken breast samples were <0.3 and 4.9±0.5 log CFU/g, respectively.
- The pH of control samples after fryer browning and frozen storage was 6.19. Compared to the control, formulation of products with CAA (0.5 or 1.0%) or PAA (0.3 or 0.5%) lowered (P<0.05) the pH of products to 5.52 to 6.02.
- The water activity of samples from all treatments after fryer browning ranged from 0.971 to 0.979.
- Samples from all treatments evaluated in this study reached an average maximum geometric center temperature of 35.7±1.8°C during the 15 s browning period in the deep fryer (Figure 4).

<u>Study 4</u>: The effect of various concentrations of caprylic acid on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

- *Salmonella* counts of inoculated control samples after oven browning and after frozen storage (-20°C, 7 days) were 0.5 ($P \geq 0.05$) and 1.0 ($P < 0.05$) log CFU/g lower than initial levels (4.9±0.2 log CFU/g).
- Compared to the control, all tested CAA concentrations effectively ($P < 0.05$) reduced *Salmonella* and total bacterial counts in the raw ground chicken with salt and phosphate mixture (i.e., approximately 15 min after addition of the antimicrobial solution). Within each CAA treatment, further reductions in *Salmonella* counts were obtained after oven browning of the breaded products and/or after 7 days of frozen storage.
- Total pathogen reductions for samples treated with CAA (0.25 to 1.0%) were 2.9 to >4.5 log CFU/g in the final, frozen products. More specifically, reductions of 2.9, 4.0, 4.5, and >4.5 log CFU/g were obtained for samples treated with 0.25, 0.5, 0.75 or 1.0% CAA, respectively. In products formulated with 0.75 or 1.0% CAA, initial counts (4.8 log CFU/g) were reduced ($P < 0.05$) to at/below the detection limit (<0.3 log CFU/g).
- Uninoculated chicken breast samples had total bacterial counts of 4.0±0.5 log CFU/g, and hydrogen sulfide-producing *Salmonella* populations were not detected (<0.3 log CFU/g) in any of the samples.
- The only concentrations of CAA that significantly ($P < 0.05$) reduced the pH of the frozen, breaded chicken products, compared to the untreated control (pH 6.06), were 0.75 and 1.0% CAA (pH 5.89 and 5.82, respectively).
- The water activity was not ($P \geq 0.05$) affected by the addition of CAA to product formulations, irrespective of concentration. Water activities ranged from 0.969 to 0.973.
- The average temperature of the geometric center of samples from all tested treatments, including the control, reached a maximum of 50.8±3.9°C during oven browning (208°C, 15 min) (Figure 5).

<u>Study 5</u>: The effect of various concentrations of carvacrol on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

- *Salmonella* counts of inoculated (4.9±0.2 log CFU/g) control samples were reduced ($P \geq 0.05$) by 0.8 log CFU/g after frozen storage (-20°C, 7 days) of oven-browned, breaded products.
- Pathogen counts of samples analyzed approximately 15 min after treatment with 0.3, 0.4, or 0.5% CAR were 2.2, >3.4, and >4.4 log CFU/g, respectively, lower ($P < 0.05$) than counts of untreated (control) samples.
- Total *Salmonella* reductions of 3.4, >4.4, and >4.3 log CFU/g were obtained in frozen, oven-browned, breaded products formulated with 0.3, 0.4, or 0.5% CAR, respectively.
- *Salmonella* (i.e., hydrogen sulfide-producing populations) and total bacterial counts of uninoculated chicken breast samples were <0.3 and 3.4±0.2 log CFU/g, respectively.
- The pH values of the final, frozen products were in the range of 6.06 to 6.18; no differences ($P \geq 0.05$) were found between the CAR-treated samples and the control.
- The water activity of oven-browned, breaded chicken samples formulated with 0.5% CAR (water activity of 0.977) was higher ($P < 0.05$) than that of the control (water activity of 0.974) or samples formulated with 0.3 or 0.4% CAR (water activity of 0.975).
- The average temperature of the geometric center of samples from all treatments reached a maximum of 47.2±3.9°C during the 15 min oven browning period (Figure 6).

<u>Study 6</u>: The effect of various concentrations of ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

- Inoculated levels (4.9±0.1 log CFU/g) of *Salmonella* in control samples were reduced ($P<0.05$) to 4.3 and 3.7 log CFU/g (i.e., total reductions of 0.6 and 1.2 log CFU/g) after oven browning and after frozen storage, respectively.
- The lowest concentration of POL needed to significantly ($P<0.05$) reduce *Salmonella* counts, compared to the control, in the raw ground chicken with salt and phosphate mixture (i.e., approximately 15 min after addition of the antimicrobial solution) was 0.25%. However, after oven browning and frozen storage, at least 0.5% POL was needed to significantly ($P<0.05$) lower pathogen counts of samples compared to the control.
- Overall, total reductions of *Salmonella* in the frozen, oven-browned, breaded products treated with 0.125, 0.25, 0.5, or 1.0% POL were 1.8, 1.4, 2.0, and 2.3 log CFU/g, respectively. Pathogen counts of samples (after frozen storage) treated with 0.5 or 1.0% POL were lower ($P<0.05$) than the counts of control samples.
- Uninoculated chicken breast samples analyzed for natural microbial contamination levels had total bacterial counts of 4.1±0.8 log CFU/g. Hydrogen sulfide-producing *Salmonella* populations were not detected (<0.3 log CFU/g) in any of the uninoculated samples.
- Compared to the control, all tested POL concentrations resulted in an increase ($P<0.05$) in the pH of the frozen, oven-browned, breaded products.
- Water activities of samples from all POL treatments (water activities of 0.962 to 0.969), were lower ($P<0.05$) than the water activity of the control treatment (water activity of 0.974).
- Samples from all treatments evaluated in this study reached an average maximum geometric center temperature of 43.5±0.7°C during oven browning (208°C, 15 min) (Figure 7).

<u>Study 7</u>: The effect of combinations of 0.25% caprylic acid, 0.3% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

- *Salmonella* counts of inoculated control samples after oven browning and after frozen storage (-20°C, 7 days) were 0.5 and 1.1 log CFU/g lower ($P<0.05$) than initial levels (4.8±0.2 log CFU/g).
- Significant ($P<0.05$) reductions in *Salmonella* counts, compared to the control, were obtained for all tested individual and combination antimicrobial treatments, irrespective of sampling point. Pathogen counts of samples analyzed approximately 15 min after treatment with the combination of all three antimicrobials (i.e., 0.25% CAA + 0.3% CAR + 0.5% POL) were >3.0 log CFU/g lower ($P<0.05$) than counts of untreated (control) samples. In most cases, additional reductions in *Salmonella* counts were obtained after oven browning of the treated, breaded products and/or after 7 days of frozen storage.
- Total reductions of *Salmonella* in the frozen, oven-browned, breaded products treated with individual or combination antimicrobial treatments ranged from 1.6 (0.5% POL) to >4.5 (0.25% CAA + 0.3% CAR + 0.5% POL) log CFU/g. At least 4.0 log CFU/g total reductions in pathogen levels were also obtained in samples treated with 0.3% CAR, 0.25% CAA + 0.3% CAR, or 0.3% CAR + 0.5% POL.
- *Salmonella* (i.e., hydrogen sulfide-producing populations) and total bacterial counts of uninoculated chicken breast samples were <0.3 and 5.0±0.3 log CFU/g, respectively.

- Products formulated with 0.5% POL, alone, or in combination with CAA and/or CAR had higher ($P<0.05$) pH values than that of the untreated (control) product. More specifically, the pH ranged from 6.55 to 6.62 in POL-treated final products (i.e., after frozen storage), whereas the pH of the remaining product formulations, including the control, ranged from 6.15 to 6.29.
- The water activities of oven-browned products treated with 0.25% CAA + 0.3% CAR, 0.3% CAR + 0.5% POL, or 0.25% CAA + 0.3% CAR + 0.5% POL were higher ($P<0.05$) than the water activity of the control product.
- The average temperature of the geometric center of samples from all tested treatments, including the control, reached a maximum of 45.0±3.0°C during the 15 min oven browning period (Figure 8).

<u>Study 8</u>: The effect of combinations of 0.125% caprylic acid, 0.15% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

- Initial levels (5.0±0.2 log CFU/g) of *Salmonella* in inoculated control samples were reduced ($P<0.05$) by 1.4 log CFU/g in the final, frozen, oven-browned product.
- Compared to the control, all antimicrobial combination treatments evaluated reduced ($P<0.05$) pathogen counts in the raw ground chicken with salt and phosphate mixture (i.e., approximately 15 min after addition of the antimicrobial solution), with reductions ranging from 1.3 (0.15% CAR + 0.5% POL) to 2.7 (0.25% CAA + 0.3% CAR + 0.5% POL) log CFU/g.
- Within each antimicrobial treatment, further reductions ($P<0.05$) of the pathogen were generally obtained after browning and frozen storage of samples.
- Total reductions of *Salmonella* in the frozen, oven-browned, breaded products treated with combinations of two or three antimicrobials ranged from 1.7 (0.125% CAA + 0.5% POL) to >4.6 (0.25% CAA + 0.3% CAR + 0.5% POL) log CFU/g. Total pathogen reductions of at least 4.2 log CFU/g were also obtained for products formulated with 0.25% CAA + 0.3% CAR, 0.3% CAR + 0.5% POL, or 0.125% CAA + 0.15% CAR + 0.5% POL.
- *Salmonella* counts in samples (after frozen storage) of all antimicrobial treatments tested, except 0.125% CAA + 0.5% POL, were lower ($P<0.05$) than counts of the control treatment.
- Total bacterial counts of uninoculated samples were 5.0±0.3 log CFU/g, and no hydrogen sulfide-producing *Salmonella* populations were detected (<0.3 log CFU/g) on XLD agar.
- As seen in Study 7, all antimicrobial treatments that included 0.5% POL resulted in an increase ($P<0.05$) in the pH of samples, compared to the control, irrespective of sampling point. The pH of POL-containing products (after frozen storage) was 6.56 to 6.70, while the pH of all other treatments ranged from 6.20 to 6.28.
- The water activity of the control treatment after oven browning was 0.971. Water activities of products formulated with combinations of two or three antimicrobials ranged from 0.973 to 0.978.
- Samples from all treatments evaluated in this study reached an average maximum geometric center temperature of 46.0±3.9°C during oven browning (208°C, 15 min (Figure 9).

<u>Study 9</u>: The effect of combinations of 0.03125% or 0.0625% caprylic acid, 0.0375% or 0.075% carvacrol, and 0.5% ε-polylysine on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

- Inoculated levels (5.0±0.2 log CFU/g) of *Salmonella* in control samples were reduced ($P<0.05$) to 4.2 log CFU/g (i.e., total reduction of 0.8 log CFU/g) after frozen storage of oven-browned, breaded products.
- All tested antimicrobial treatments effectively ($P<0.05$) reduced pathogen counts of the raw ground chicken with salt and phosphate mixture compared to the untreated control. Specifically, counts were 0.5 (0.0625% CAA + 0.075% CAR) to 1.3 (0.0625% CAA + 0.075% CAR + 0.5% POL) log CFU/g lower ($P<0.05$) than those of the control. Within each antimicrobial treatment, oven browning and/or storage at -20°C (7 days) resulted in further pathogen reductions, except in products formulated with 0.03125% CAA + 0.0375% CAR + 0.5% POL.
- The highest total reduction (2.4 log CFU/g) of *Salmonella* in the frozen, oven-browned, breaded product was obtained in samples treated with 0.0625% CAA + 0.075% CAR + 0.5% POL. The remaining tested antimicrobial combination treatments reduced initial *Salmonella* levels by 1.3 to 2.0 log CFU/g.
- Uninoculated chicken breast samples had total bacterial counts of 3.5±0.3 log CFU/g, and no hydrogen sulfide-producing *Salmonella* populations were detected (<0.3 log CFU/g) in any of the samples.
- The pH of final, frozen products ranged from 6.11 (0.0625% CAA + 0.075 CAR) to 6.59 (0.075% CAR + 0.5% POL). As seen in previous studies, products formulated with 0.5% POL had higher ($P<0.05$) pH values than that of the untreated control.
- The water activity of samples from all treatments after oven browning was in the range of 0.970 to 0.977.
- The average temperature of the geometric center of samples from all treatments reached a maximum of 42.5±3.9°C during the 15 min oven browning period (Figure 10).

C. Study addressing Objective 4

<u>Study 10</u>: Evaluation of the organoleptic properties of fully cooked, breaded chicken products treated with selected antimicrobials.

- As indicated in the Materials and Methods, the sensory analysis was conducted by a panel comprised of 5 to 6 individuals, and the overall purpose of this work was to obtain preliminary data on the organoleptic properties of individual and combination antimicrobial treatments identified under Studies 2 to 9 to significantly ($P<0.05$) reduce *Salmonella* contamination in the frozen, not-ready-to-eat breaded chicken product.
- Under the conditions of the study, products formulated with 0.5% POL, alone, were found organoleptically acceptable by the sensory panel compared to an untreated control (no antimicrobial ingredients); all other tested treatments, except 0.06% CAA, were found less desirable. The 0.06% CAA treatment was included in the sensory analysis as a control treatment (i.e., it was not one of the treatments selected based on microbiological data obtained in studies conducted under Objectives 2 and 3); therefore, the antimicrobial effects against *Salmonella* of 0.06% CAA are not known at this time.
- As described in the Materials and Methods, product formulations evaluated in the sensory analysis were given a score of 0 to 5 (0 = same as control; 1 = very weakly different to the control; 2 = weakly different to the control; 3 = clearly different to the control; 4 =

strongly different to the control; and 5 = very strongly different to the control) for intensity of odor, taste, and mouth feel as compared to the control (no antimicrobial ingredients). Mean scores, based on this 0 to 5 scale, of selected evaluated treatments were:
  - 0.5% POL: 0.5±0.5 (odor), 1.5±0.8 (taste), and 1.8±1.2 (mouth feel)
  - 0.06% CAA (control treatment): 0.6±0.5 (odor), 1.9±0.5 (taste), and 0.8±1.0 (mouth feel)
  - 0.06% CAA + 0.08% CAR: 2.0±1.5 (odor), 2.7±1.4 (taste), and 1.4±1.8 (mouth feel)
  - 0.15% CAR + 0.5% POL: 1.8±1.2 (odor), 3.2±1.4 (taste), and 1.6±2.3 (mouth feel)
  - 0.06% CAA + 0.08% CAR + 0.5% POL: 2.2±1.2 (odor), 4.0±0.6 (taste), and 1.6±2.3 (mouth feel)
  - 0.25% CAA + 0.5% POL: 3.8±1.0 (odor), 4.4±0.5 (taste), and 1.8±1.9 (mouth feel)
- Panelists described products formulated with CAA as having a "goat cheese taste", and products formulated with CAR as being "spicy" or having a "strong herbal flavor".

D. Additional studies not included in the original proposal

<u>Study 11</u>: The effect of product dimensions and surface browning method on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products treated with various antimicrobials.

- Reductions in *Salmonella* counts and total bacterial counts in surface-browned, breaded chicken products after frozen storage were found to vary depending on the product dimensions, surface browning method, and antimicrobial treatment.
- For inoculated (4.9±0.2 log CFU/g) control samples, total pathogen reductions of 0.7 and 3.8 log CFU/g were obtained in oven-browned 9 × 5 × 3 cm and 9 × 2.5 × 2 cm samples, respectively. For samples browned in the deep fryer, total pathogen reductions of 0.6 log CFU/g were obtained, irrespective of product size.
- Irrespective of the antimicrobial treatment and browning method, the highest total reductions (at least 4.6 log CFU/g) of *Salmonella* counts were obtained in 9 × 2.5 × 2 cm samples browned in the oven.
- Irrespective of the antimicrobial treatment, oven browning resulted in higher ($P<0.05$) total reductions of *Salmonella* counts (reductions of >4.6 log CFU/g) than browning in the deep fryer (reductions of 1.6 to 2.5 log CFU/g), but only for 9 × 2.5 × 2 cm samples.
- Irrespective of the browning method and product dimensions, formulation of products with the antimicrobial treatments reduced initial *Salmonella* levels by 1.6 to >4.6 (0.0625% CAA + 0.075% CAR), 1.7 to >4.6 (0.25% CAA + 0.5% POL), 2.5 to >4.6 (0.15% CAR + 0.5% POL), and 1.5 to >4.6 (0.0625% CAA + 0.075% CAR + 0.5% POL) log CFU/g. Compared to the control, products treated with 0.15% CAR + 0.5% POL generally had the lowest ($P<0.05$) numbers of pathogen survivors in the final product (after frozen storage).
- Irrespective of browning method or product dimensions, pH values of the control product (after frozen storage) and products formulated with 0.0625% CAA + 0.075% CAR (pH 5.92 to 6.01) were not ($P\geq0.05$) different. The pH values of products formulated with 0.25% CAA + 0.5% POL, 0.15% CAR + 0.5% POL, or 0.0625% CAA + 0.075% CAR + 0.5% POL (pH 6.20 to 6.33) were higher ($P<0.05$) than the pH of the control treatments.

- The average temperature of the geometric center of 9 × 5 × 3 cm and 9 × 2.5 × 2 cm, oven-browned samples from all treatments reached a maximum of 46.0±3.0°C and 62.4±4.0°C, respectively, during the 15 min browning period (Figure 11).
- The average temperature of the geometric center of 9 × 5 × 3 cm and 9 × 2.5 × 2 cm, fryer-browned samples from all treatments reached a maximum of 31.7±2.6°C and 35.0±1.1°C, respectively, during the 15 s browning period (Figure 12).
- Samples browned in the deep fryer had a more attractive "golden-brown" color than samples browned in the oven (Figure 13).
- The "browning effect" obtained by a specific browning method was the same regardless of the dimensions of the samples (Figures 14 and 15).

<u>Study 12</u>: The effect of lauric arginate, and its combinations with caprylic acid, carvacrol, and/or ε-polylysine, on reductions of *Salmonella* contamination in frozen, surface-browned, breaded chicken products.

- Inoculated levels (4.9±0.1 log CFU/g) of *Salmonella* in control samples were reduced to 4.3 and 4.0 log CFU/g (i.e., total reductions of 0.6 and 0.9 log CFU/g) in frozen, breaded not-ready-to-eat products surface-browned in the deep fryer (190°C, 15 s) or oven (208°C, 15 min), respectively. No significant ($P \geq 0.05$) differences in pathogen or total bacterial population survivors were obtained between the two browning methods for control samples.
- In products formulated with 0.02% LAE, total pathogen reductions of 0.8 and 1.0 log CFU/g were obtained in samples surface-browned in the deep fryer or oven, respectively, and stored at -20°C for 7 days. *Salmonella* counts of products formulated with 0.02% LAE were not ($P \geq 0.05$) different than counts of control samples.
- For all other antimicrobial treatments, total reductions of *Salmonella* after frozen storage of products ranged from 1.1 (0.02% LAE + 0.5% POL, fryer browning) to 1.8 (0.03% CAA + 0.04% CAR + 0.5% POL, oven browning) log CFU/g.
- Compared to the control, all the tested antimicrobial treatments, except 0.02% LAE, significantly ($P < 0.05$) reduced *Salmonella* counts in the final product, irrespective of browning method.
- Irrespective of browning method, *Salmonella* counts of products formulated with 0.02% LAE + 0.5% POL were not ($P \geq 0.05$) different than counts of samples formulated with 0.5% POL alone.
- Additionally, formulation of products with all four tested antimicrobials (i.e., 0.03% CAA + 0.04% CAR + 0.5% POL + 0.02% LAE) did not ($P \geq 0.05$) enhance pathogen reductions obtained in samples treated with 0.03% CAA + 0.04% CAR or 0.03% CAA + 0.04% CAR + 0.5% POL.
- *Salmonella* counts of oven-browned products formulated with 0.02% LAE + 0.5% POL, 0.03% CAA + 0.04% CAR + 0.5% POL, or 0.03% CAA + 0.04% CAR + 0.5% POL + 0.02% LAE were 0.3 to 0.5 log CFU/g lower ($P < 0.05$) than corresponding products surface-browned in the deep fryer.
- Within each browning method, similar ($P \geq 0.05$) pH values were obtained for the control and samples formulated with 0.02% LAE or 0.03% CAA + 0.04% CAR (fryer-browned: pH 6.05 to 6.13; oven-browned: pH 6.13 to 6.19) after frozen storage. Higher ($P < 0.05$) pH values, compared to the control, were obtained for products formulated with the remaining antimicrobial treatments, irrespective of surface browning method (pH 6.46 to 6.56).

- The average temperature of the geometric center of samples from all treatments browned in the deep-fryer (190°C, 15 s) or oven (208°C, 15 min) reached a maximum of 26.9±6.1°C and 44.6±4.5°C, respectively (Figures 16 and 17).

VIII. CONCLUSIONS

Under the conditions of the above studies, the main findings were:

A. Study addressing Objective 1

- This screening study (Study 1) identified several antimicrobials that effectively reduced *Salmonella* contamination in raw chicken breast portions. At least 4.5 log CFU/g reductions were obtained in samples treated with 2.0% allyl isothiocyanate, 1.0% caprylic acid, 0.3 or 0.5% carvacrol, 0.5% oregano oil, or 0.5% peracetic acid. Also, pathogen counts were reduced by 2.5 log CFU/g in samples treated with 0.25% ε-polylysine.
- Citric acid, malic acid, sodium lactate, sodium citrate, and grapefruit terpenes, tested up to a concentration of 2.0%, had limited or no effect against *Salmonella*.
- Based on the findings of this study, four antimicrobials were selected for further evaluation in a process simulating the manufacture of a frozen, not-ready-to-eat breaded chicken product (under Objectives 2 and 3). These antimicrobials were carvacrol (the most effective essential oil), caprylic acid and peracetic acid (the two most effective acids), and ε-polylysine.

B. Studies addressing Objectives 2 and 3

- Several studies (Studies 2 to 9) were conducted under Objectives 2 and 3 to evaluate the antimicrobial effects of different concentrations of carvacrol, caprylic acid, peracetic acid, and ε-polylysine, applied individually or in combinations of two or three, against *Salmonella* during production and frozen storage of a not-ready-to-eat breaded chicken product.
- Total reductions of *Salmonella* obtained in untreated (control, no antimicrobial ingredients) surface-browned (by oven baking or flash frying), breaded chicken products after frozen storage (-20°C, 7 days) were 0.8 to 1.4 log CFU/g (Studies 2 to 9).
- Total reductions of *Salmonella* in products (after frozen storage) formulated with the lowest tested concentrations of single or combination antimicrobial treatments that effectively ($P<0.05$) reduced pathogen contamination compared to the untreated control product were (Studies 2 to 9):
  - 0.25% caprylic acid: 2.5 to 2.9 log CFU/g
  - 0.3% carvacrol: 3.4 to at least 4.0 (surface-browned by oven baking) and 4.1 (surface-browned by flash frying) log CFU/g
  - 0.3% peracetic acid: 2.1 (surface-browned by oven baking) and 1.6 (surface-browned by flash frying) log CFU/g
  - 0.5% ε-polylysine: 1.6 to 2.0 log CFU/g
  - 0.0625% caprylic acid + 0.075% carvacrol: 1.8 log CFU/g
  - 0.0625% caprylic acid + 0.5% ε-polylysine: 1.8 log CFU/g
  - 0.075% carvacrol + 0.5% ε-polylysine: 2.0 log CFU/g
  - 0.03125% caprylic acid + 0.0375% carvacrol + 0.5% ε-polylysine: 1.3 log CFU/g
- Use of peracetic acid in product formulations was discontinued after Studies 2 and 3 were completed because it resulted in product discoloration and an offensive odor, thus making

this ingredient unsuitable for use in this type of application, at least under the conditions described in this report, and the concentrations evaluated (0.3 and 0.5%).

C. Study addressing Objective 4

- Based on the preliminary sensory evaluation conducted (Study 10), products formulated with 0.5% ε-polylysine, alone, were found to be organoleptically acceptable by a sensory panel (comprised of 5 to 6 individuals), compared to an untreated control (no antimicrobial ingredients), while products formulated with caprylic acid and/or carvacrol were less desirable. One of the control treatments (i.e., 0.06% caprylic acid) included in the sensory evaluation was also found to be organoleptically acceptable by the panelists; however, the antimicrobial effects against *Salmonella* of this treatment have not been determined.
- If there is interest by the industry to use any of the tested antimicrobials in product formulations, additional sensory evaluations will have to be conducted to determine appropriate usage levels so as not to affect product sensory properties. Levels of caprylic acid and/or carvacrol could be reduced depending on the targeted level of reduction of *Salmonella* contamination in not-ready-to-eat breaded chicken products, and/or they could be used in combination with additional antimicrobials.

D. Additional studies not included in the original proposal

- Results of Study 11 indicated that, in addition to antimicrobial treatment, product dimensions and/or surface browning method affected reductions of *Salmonella* in frozen, not-ready-to-eat breaded chicken products.
  - Oven browning of 9 × 2.5 × 2 cm samples resulted in higher ($P<0.05$) reductions of *Salmonella* than oven browning of 9 × 5 × 3 cm samples.
  - Product dimensions did not ($P\geq0.05$) affect pathogen reductions in samples surface-browned by flash frying.
  - Higher ($P<0.05$) reductions of *Salmonella* were obtained by oven browning than by flash frying, but only for 9 × 2.5 × 2 cm samples.
  - The microbiological findings of this study can most likely be partly explained by the differences in the maximum temperature reached in the geometric center of products during oven browning or flash frying.
- Results of Study 12 showed that 0.02% lauric arginate was not ($P\geq0.05$) effective against *Salmonella* when tested on its own, and it did not ($P\geq0.05$) enhance the antimicrobial activity of 0.5% ε-polylysine and/or 0.03% caprylic acid + 0.04% carvacrol.
- The combination treatment, 0.03% caprylic acid + 0.04% carvacrol, effectively ($P<0.05$) reduced *Salmonella* contamination by 1.2 (fryer-browned) and 1.5 (oven-browned) log CFU/g in the final product. These concentrations of caprylic acid and carvacrol were not part of the sensory evaluation conducted under Study 10; thus, additional work is needed to determine whether these levels (i.e., 0.03% caprylic acid + 0.04% carvacrol) would be more organoleptically acceptable than double the concentrations (i.e., 0.06% caprylic acid + 0.08% carvacrol) that were evaluated in the sensory analysis.

IX. RECOMMENDATIONS FOR FUTURE RESEARCH

Recommendations for future research related to this project include:

- Determining the effect of various post-retail manipulations of the products on *Salmonella*, simulating consumer handling of the products, e.g., thawing, undercooking, or microwave cooking of products treated with various antimicrobials.
- Testing of various other antimicrobials against *Salmonella* in frozen, browned, breaded chicken products, such as blends of organic acid salts, lactic acid, sodium diacetate, and mono- and diglycerides, potassium diacetate, or other commercially available products such as Opti.Form PD Plus (PURAC), which has been tested against acid-stressed *Salmonella* in chicken patties.
- Studies 4-9 showed that antimicrobial treatments comprised of combinations of caprylic acid and carvacrol resulted in significant reductions of *Salmonella.* On the other hand, the preliminary sensory analysis conducted under Study 10 found these treatments to be less desirable compared to an untreated control. Further studies are needed to evaluate consumer acceptability of these antimicrobials in not-ready-to-eat breaded chicken products. Levels of caprylic acid and/or carvacrol could be reduced depending on the targeted level of reduction of *Salmonella* contamination in such products, and/or they could be used in combination with additional antimicrobials.

X. PRESENTATIONS AND PUBLICATIONS

At this time, two abstracts (see Appendix) have been submitted and accepted for poster presentations at upcoming scientific meetings; one at the Annual Meeting of the Institute of Food Technologists (June 11-14, 2011; New Orleans, LA), and one at the Annual Meeting of the International Association for Food Protection (July 31-August 3, 2011; Milwaukee, WI).

The abstracts present the majority of data obtained under Objectives 2 and 3. The findings of the additional studies conducted (Studies 11 and 12) will be presented in the near future. Additionally, all data will be prepared for publication in scientific journals.

XI. REFERENCES

Currie, A., L. McDougall, J. Aramini, C. Gaulin, R. Ahmed, and S. Isaacs. 2008. Frozen chicken nuggets and strips and eggs are leading risk factors for *Salmonella* Heidelberg infections in Canada. Epidemiol. Infect. 133:809-816.

Kenny, B., R. Hall, and S. Cameron. 1999. Consumer attitudes and behaviors – key risk factors in an outbreak of *Salmonella* Typhimurium phage type 12 infection sourced to chicken nuggets. Aust. N.Z. J. Public Health 23:164-167.

MacDougall, L., M. Fyfe, L. McIntyre, A. Paccagnella, K. Cordner, A. Kerr, and J. Aramini. 2004. Frozen chicken nuggets and strips – a newly identified risk factor for *Salmonella* Heidelberg infection in British Columbia, Canada. J. Food Prot. 67:1111-1115.

Scallan, E., R.M. Hoekstra, F.J. Angulo, R.V. Tauxe, M.-A. Widdowson, S.L. Roy, J.L. Jones, and P.M. Griffin. 2011. Foodborne illness acquired in the United States-major pathogens. Emerg. Infect. Dis. 17:7-15.

Smith, K.E., C. Medus, S.D. Meyer, D.J. Boxrud, F.E. Leano, C.W. Hedberg, K. Elfering, C. Braymen, J.B. Bender, and R.N. Danila. 2008. Outbreaks of salmonellosis in Minnesota (1998 to 2006) associated with frozen, microwaveable, breaded, stuffed chicken products. J. Food Prot. 71:2153-2160.

USDA/FSIS (U.S. Department of Agriculture Food Safety and Inspection Service). 2006. Labeling policy guidance: uncooked, breaded, boneless poultry products. Available at: http://www.fsis.usda.gov/pdf/labeling_policy_guidance_uncooked_breaded_boneless_poultry_products.pdf. Accessed 27 April 2011.

XII. FIGURES

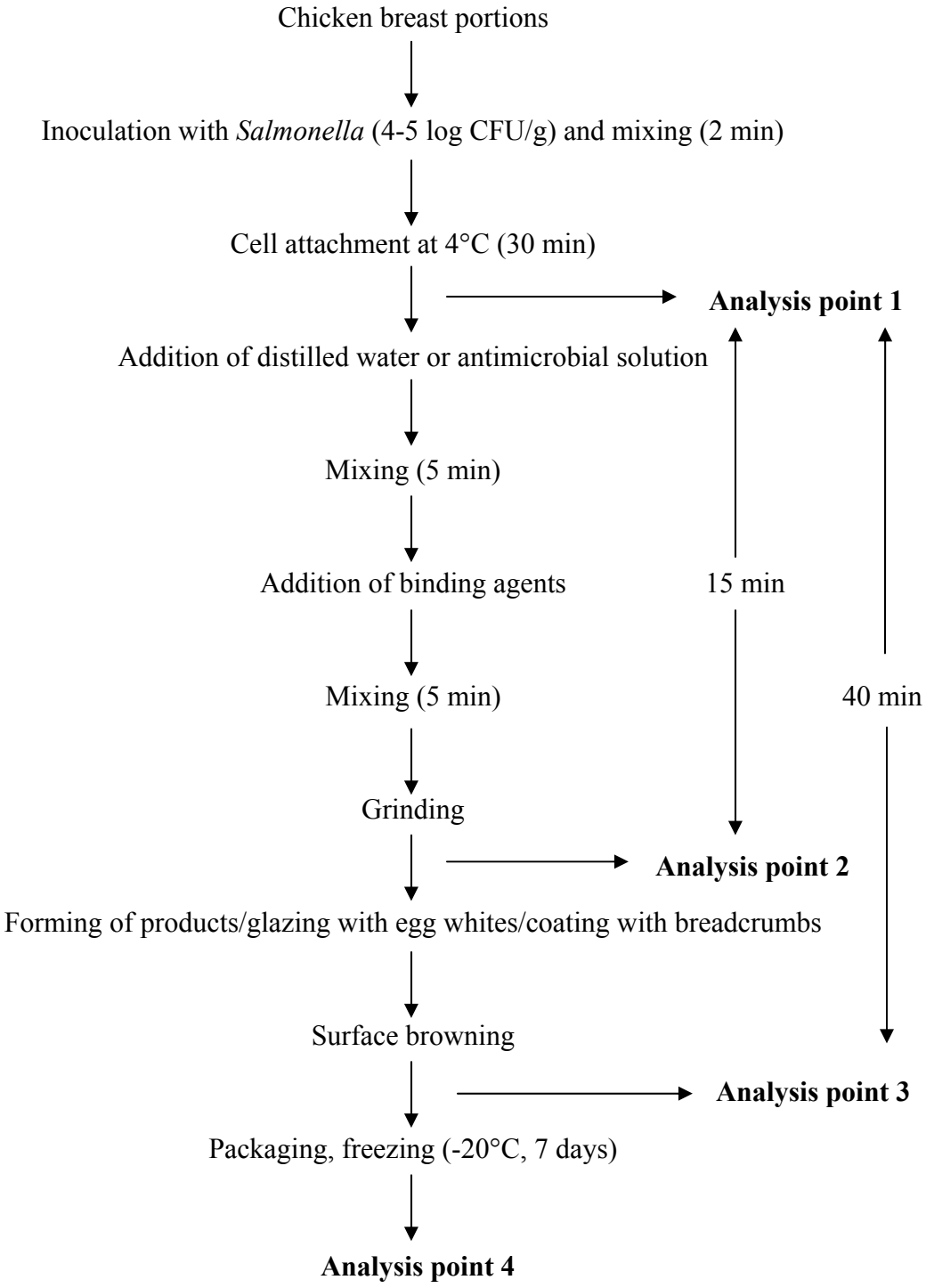

Figure 1. Flow chart showing the procedure followed for inoculation, treatment, and preparation of frozen, surface-browned, breaded chicken products, and sampling points for microbiological and physicochemical analyses of samples.

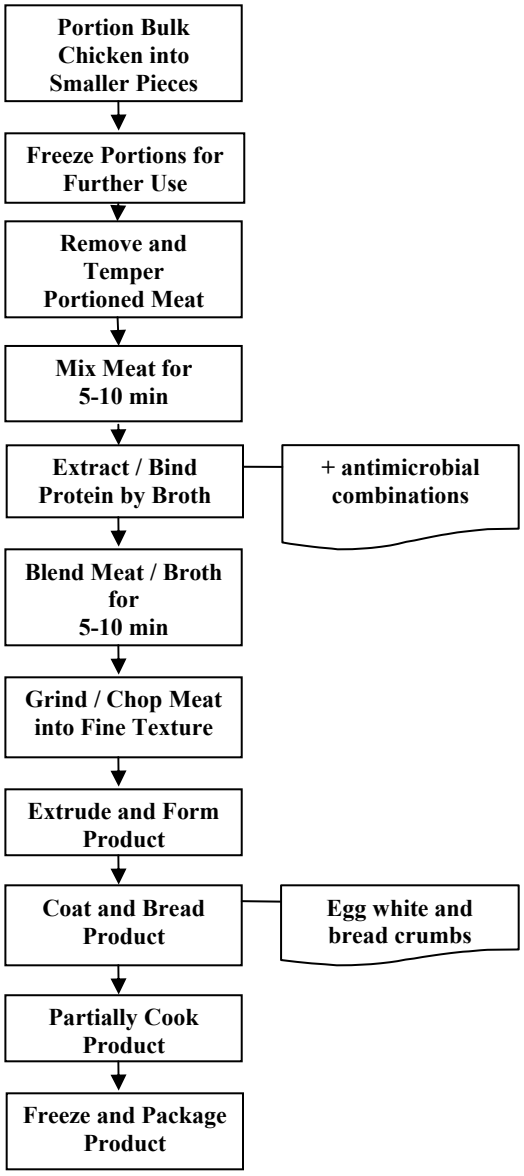

Figure 2. Procedure followed for preparation of uninoculated, frozen, surface-browned, breaded chicken products for sensory evaluation. Products were fully cooked before the sensory analysis.

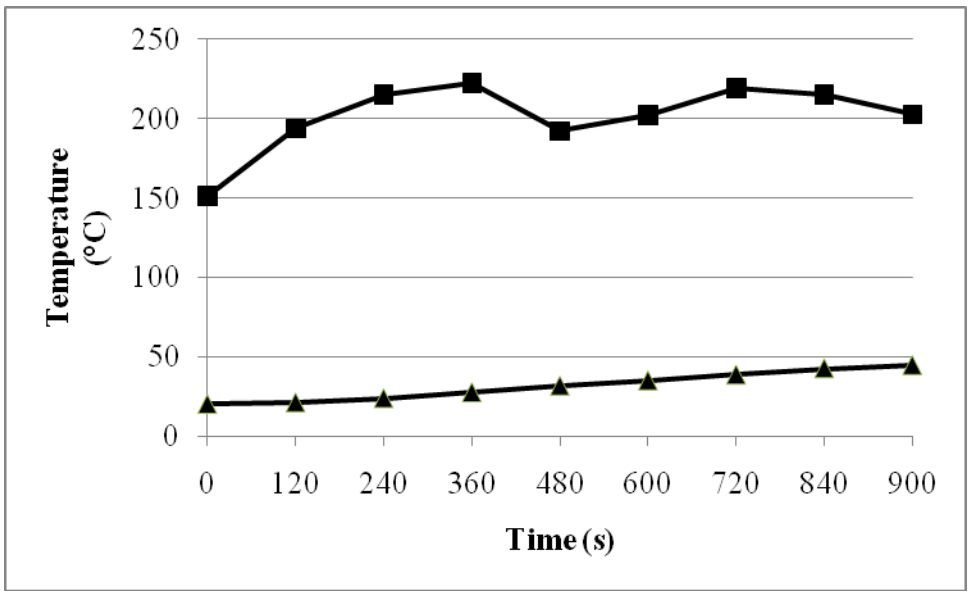

Figure 3. Changes in the temperature of the oven chamber (■) and the geometric center of samples (▲) during oven browning of breaded chicken products (Study 2).

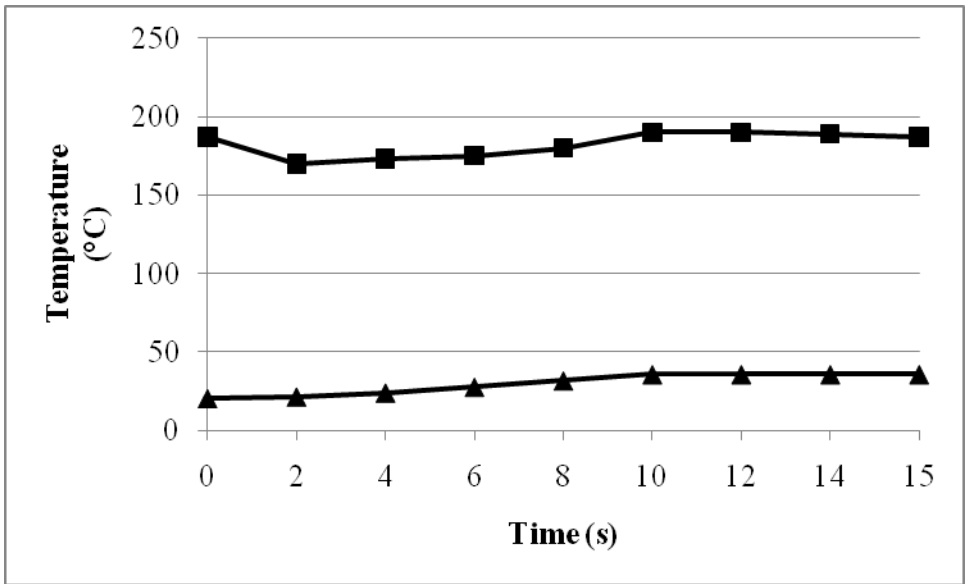

Figure 4. Changes in the temperature of the vegetable oil in the deep fryer (■) and the geometric center of samples (▲) during fryer browning of breaded chicken products (Study 3).

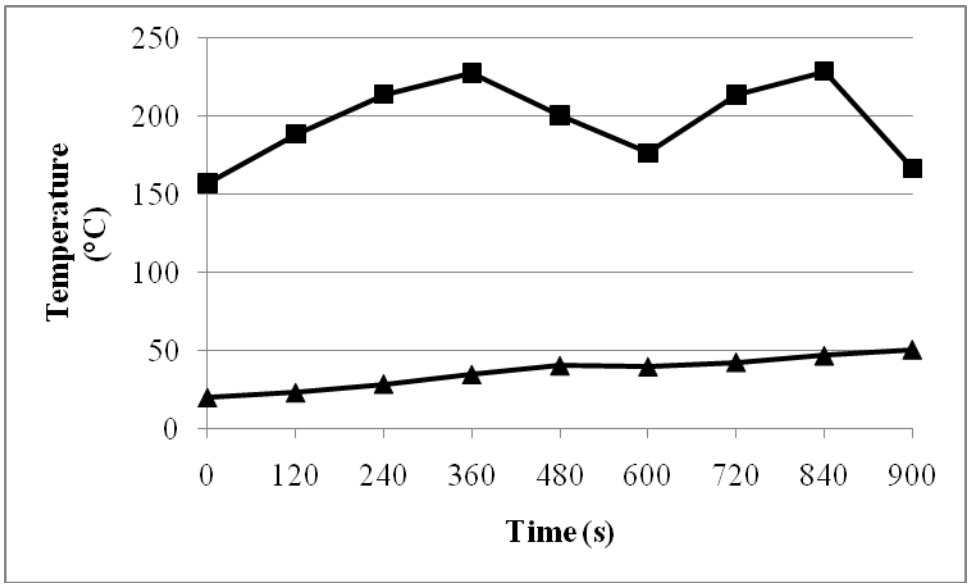

Figure 5. Changes in the temperature of the oven chamber (■) and the geometric center of samples (▲) during surface browning of breaded chicken products treated with caprylic acid (Study 4).

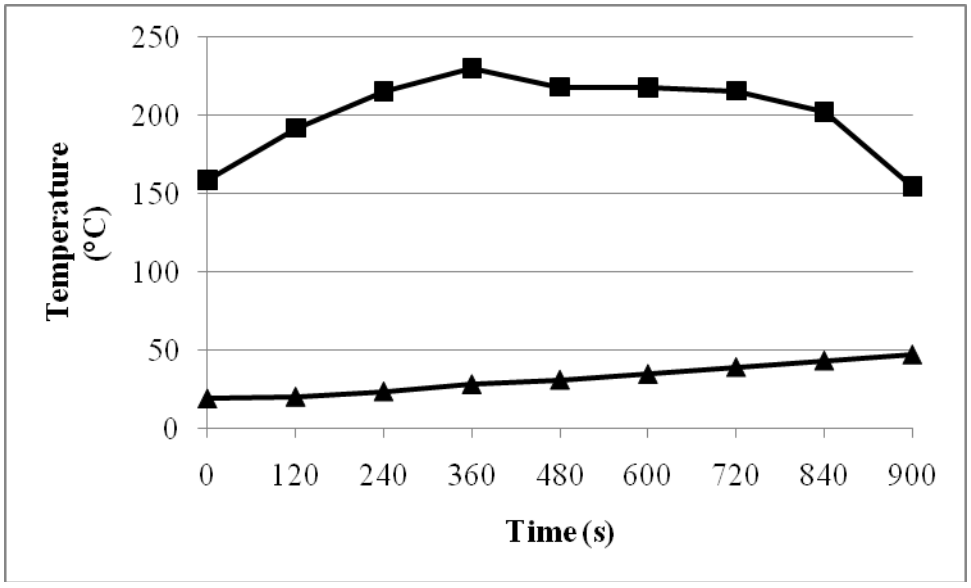

Figure 6. Changes in the temperature of the oven chamber (■) and the geometric center of samples (▲) during surface browning of breaded chicken products treated with carvacrol (Study 5).

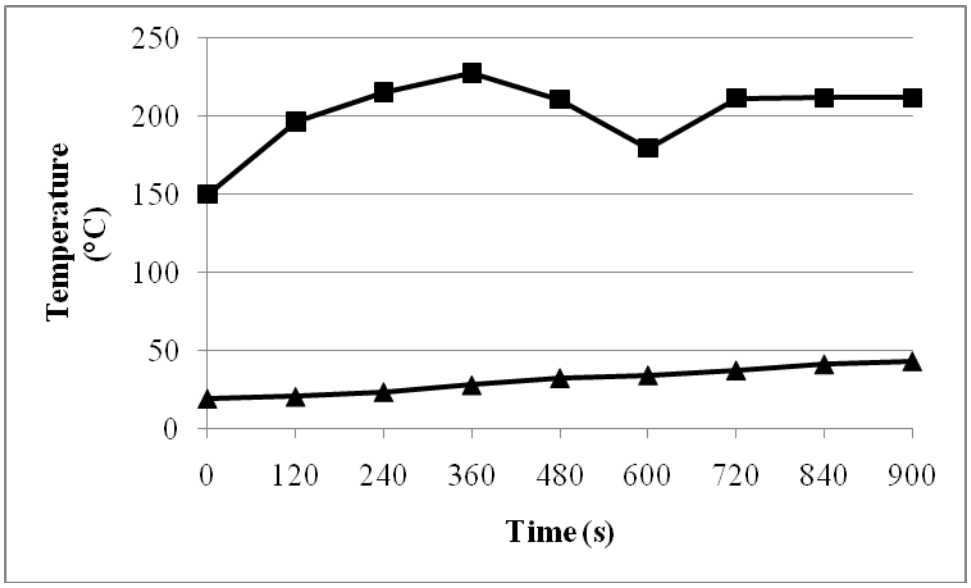

Figure 7. Changes in the temperature of the oven chamber (■) and the geometric center of samples (▲) during surface browning of breaded chicken products treated with ε-polylysine (Study 6).

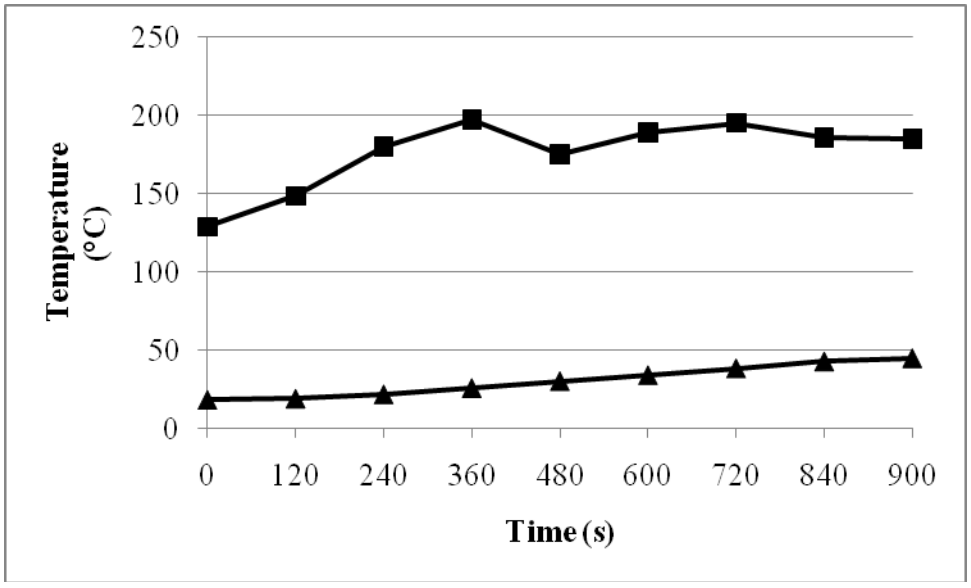

Figure 8. Changes in the temperature of the oven chamber (■) and the geometric center of samples (▲) during surface browning of breaded chicken products treated with combinations of 0.25% caprylic acid, 0.3% carvacrol, and 0.5% ε-polylysine (Study 7).

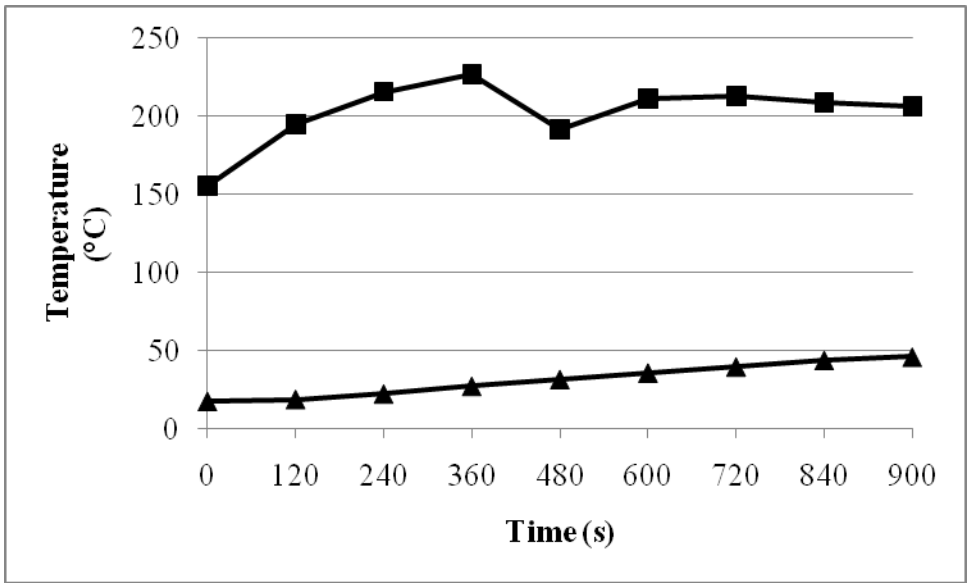

Figure 9. Changes in the temperature of the oven chamber (■) and the geometric center of samples (▲) during surface browning of breaded chicken products treated with combinations of 0.125% caprylic acid, 0.15% carvacrol, and 0.5% ε-polylysine (Study 8).

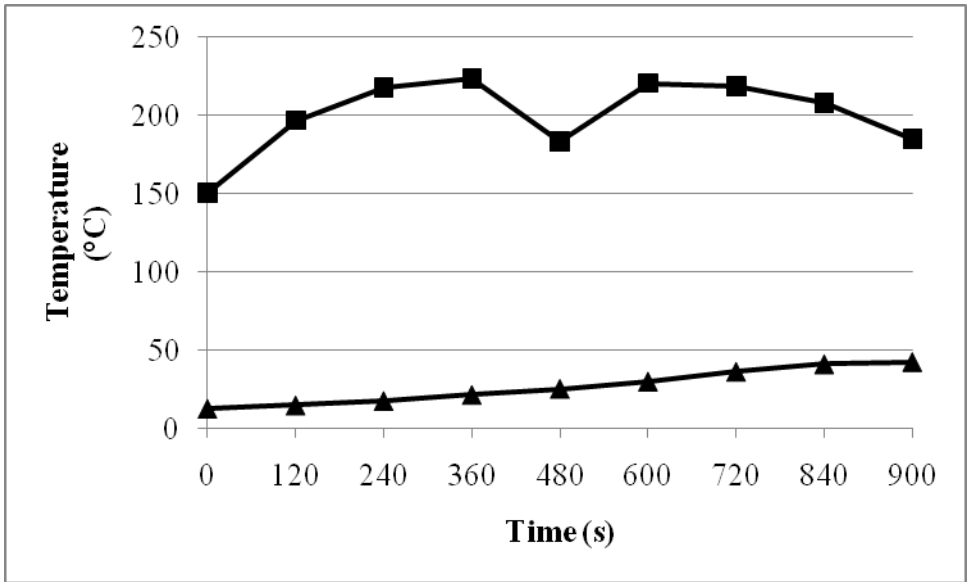

Figure 10. Changes in the temperature of the oven chamber (■) and the geometric center of samples (▲) during surface browning of breaded chicken products treated with combinations of 0.03125% or 0.0625% caprylic acid, 0.0375% or 0.075% carvacrol, and 0.5% ε-polylysine (Study 9).

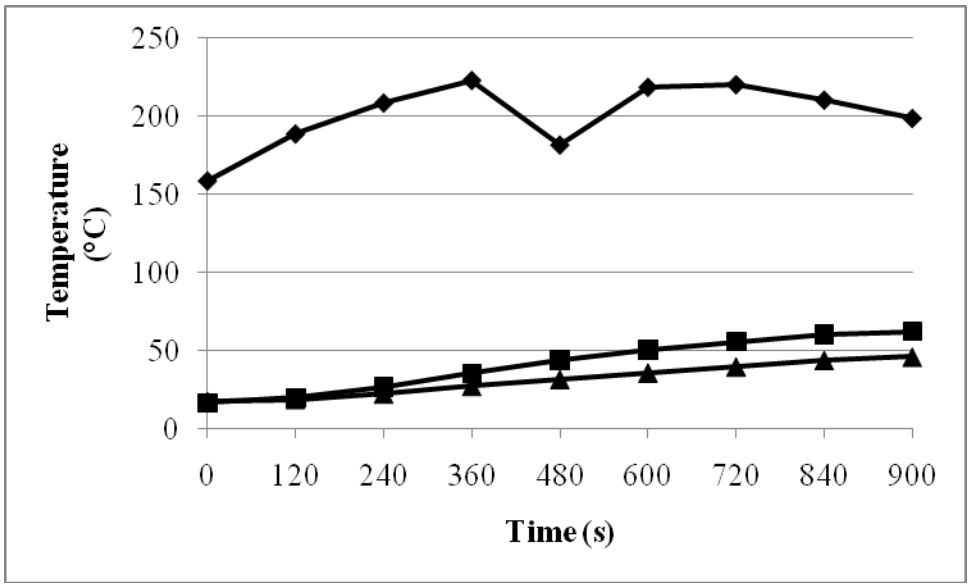

Figure 11. Changes in the temperature of the oven chamber (♦), the geometric center of 9 × 2.5 × 2 cm samples (■), and the geometric center of 9 × 5 × 3 cm samples (▲) during oven browning of breaded chicken products treated with various antimicrobials (Study 11).

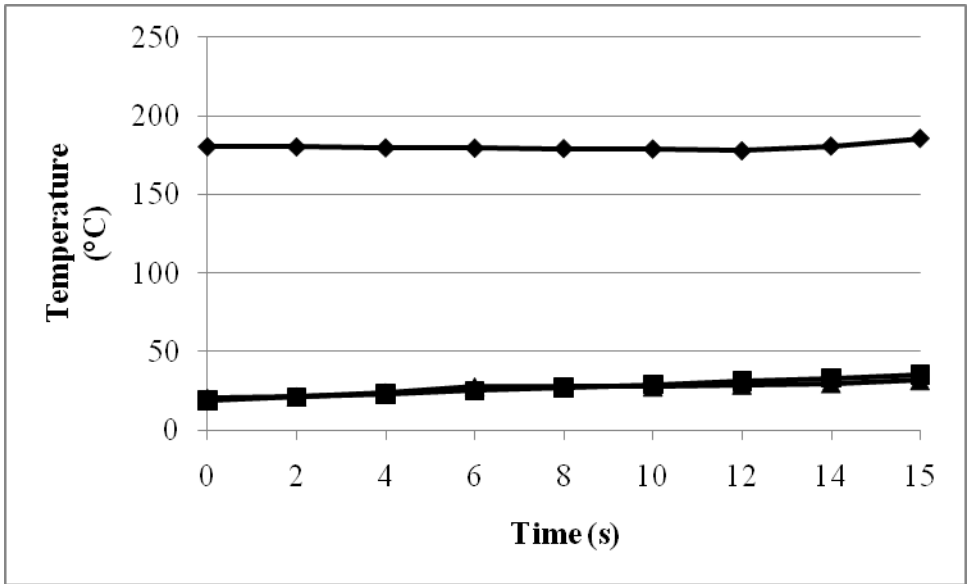

Figure 12. Changes in the temperature of the vegetable oil in the deep fryer (♦), the geometric center of 9 × 2.5 × 2 cm samples (■), and the geometric center of 9 × 5 × 3 cm samples (▲) during fryer browning of breaded chicken products treated with various antimicrobials (Study 11). The temperature profiles for the 9 × 2.5 × 2 cm and 9 × 5 × 3 cm samples are on top of each other.

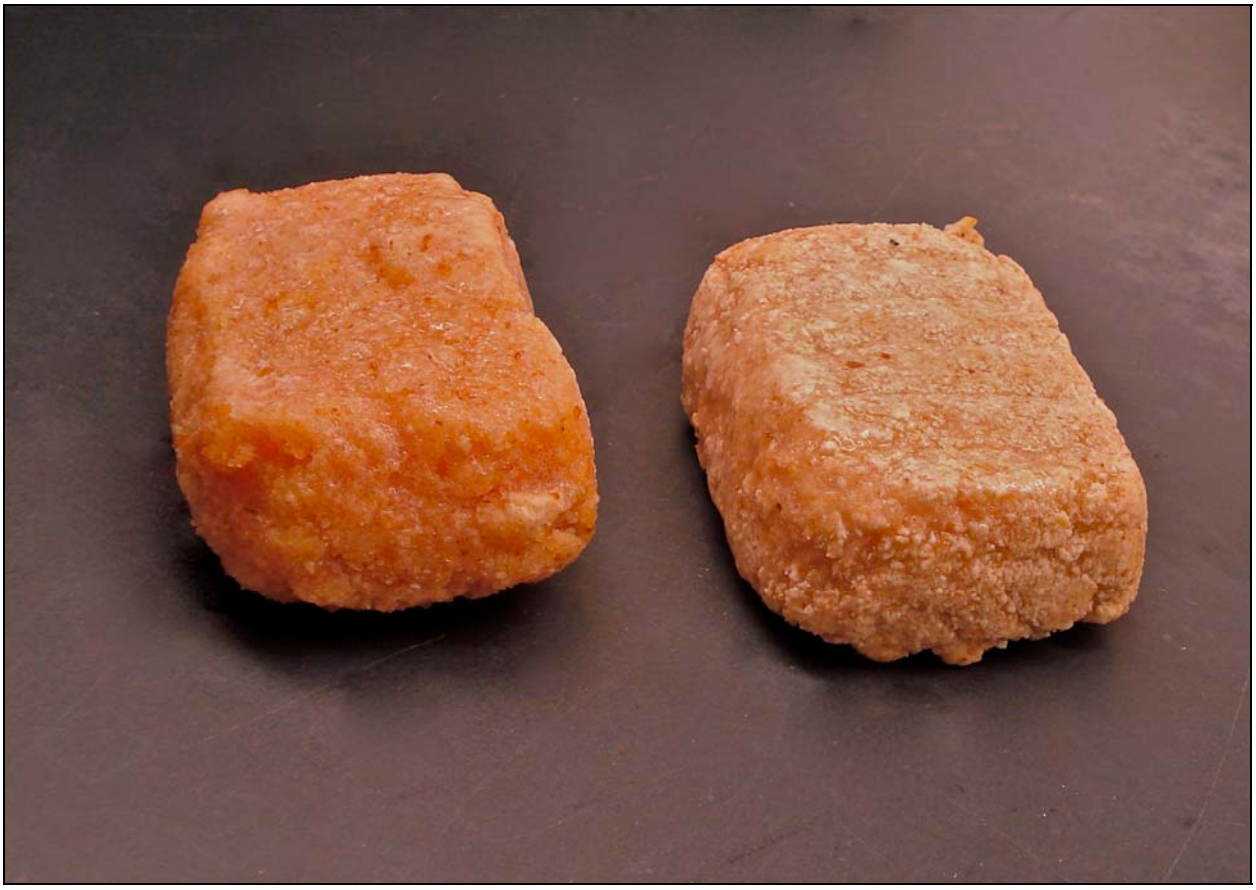

Figure 13. Samples browned in a deep fryer (190°C, 15 s; left) or in an oven (208°C, 15 min; right).

Figure 14. From left to right: 9 × 5 × 3 cm (oven-browned), 9 × 2.5 × 2 cm (oven-browned), 9 × 5 × 3 cm (fryer-browned), and 9 × 2.5 × 2 cm (fryer-browned) breaded chicken samples.

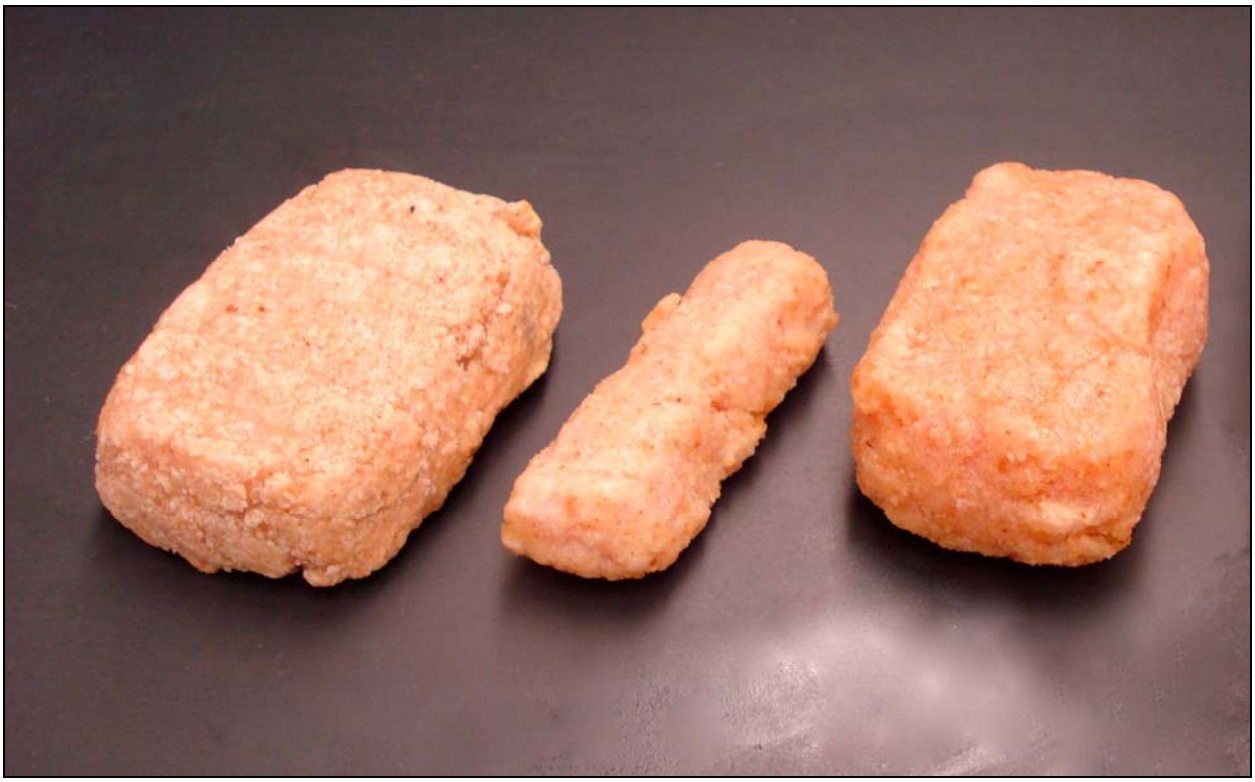

Figure 15. From left to right: 9 × 5 × 3 cm (oven-browned), 9 × 2.5 × 2 cm (oven-browned), and 9 × 5 × 3 cm (fryer-browned) breaded chicken samples.

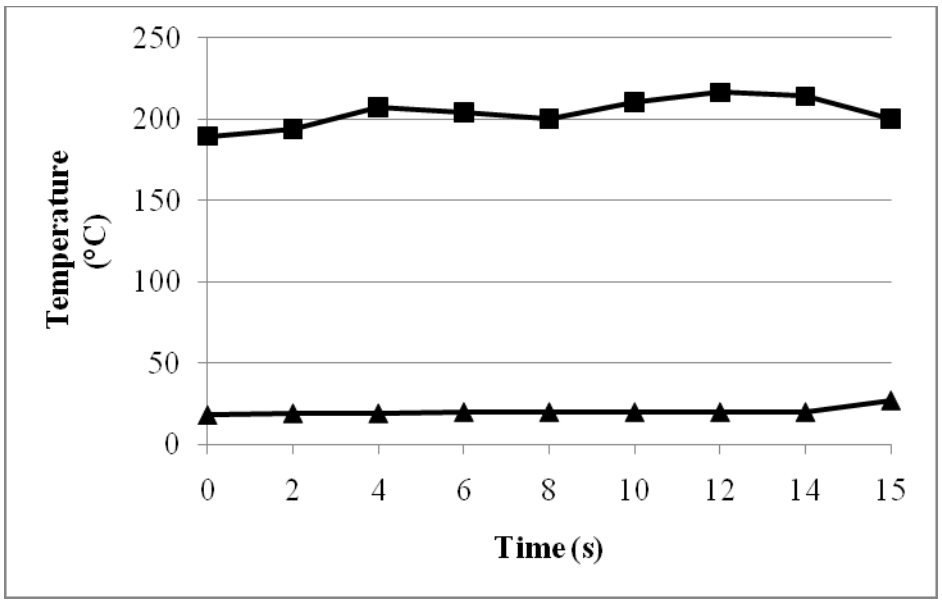

Figure 16. Changes in the temperature of the vegetable oil in the deep fryer (■) and the geometric center of samples (▲) during fryer browning of breaded chicken products (Study 12).

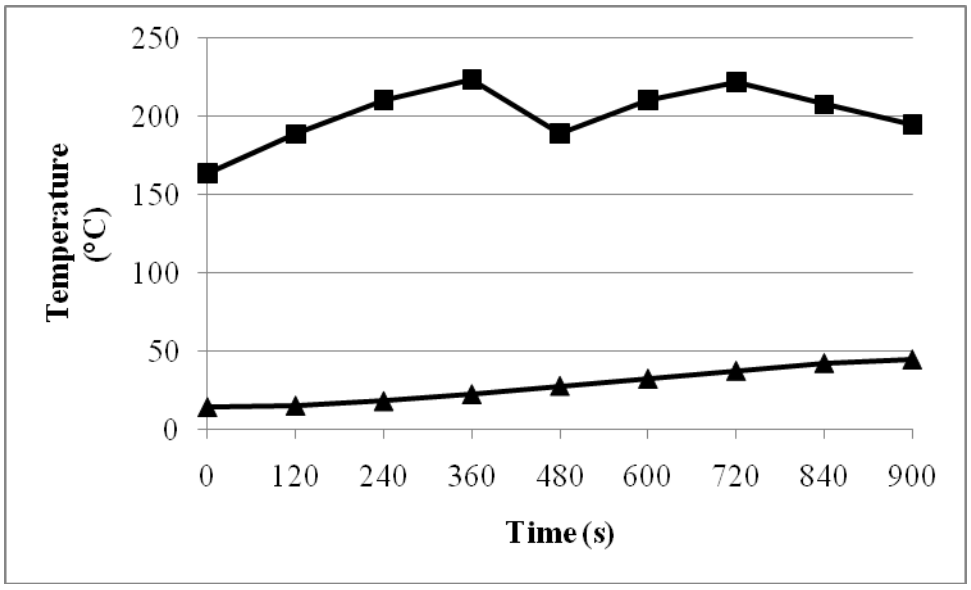

Figure 17. Changes in the temperature of the oven chamber (■) and the geometric center of samples (▲) during oven browning of breaded chicken products (Study 12).

XIII. APPENDIX

ABSTRACT ACCEPTED FOR POSTER PRESENTATION AT THE ANNUAL MEETING OF THE INSTITUTE OF FOOD TECHNOLOGISTS (JUNE 11-14, 2011; NEW ORLEANS, LA)

Title: Use of Antimicrobials to Reduce *Salmonella* Contamination in Heated for External Browning, But Uncooked, Frozen Breaded Chicken Meat Products

Authors: Galatios D. Moschonas, Ifigenia Geornaras, Jarret Stopforth, Keith E. Belk, Dale R. Woerner, Gary C. Smith, and John N. Sofos

Justification: Surface-browned, uncooked, frozen breaded chicken meat products may appear ready-to-eat, but in fact are raw; such products have been identified as sources of salmonellosis outbreaks.
Objective: This study evaluated the effect of four antimicrobials on inoculated *Salmonella* during manufacture of a surface-browned, uncooked, frozen breaded chicken meat product.
Methods: Fresh chicken breast meat portions (5×5×5 cm; 2 kg) were inoculated (5 log CFU/g) with *Salmonella* (20 ml; 7-strain mixture) followed by mixing with 20 ml of distilled water (control), caprylic acid (CAP; 0.5 and 1.0%), carvacrol (CAR; 0.3 and 0.5%), peracetic acid (PAA; 0.3 and 0.5%) or ε-polylysine (POL; 0.125 and 0.25%). Sodium chloride (1.2%) and sodium tripolyphosphate (0.3%) were added to the meat (5% total moisture enhancement level), and then ground and formed into 9×5×3 cm portions. Samples were breaded and browned by deep-frying in vegetable oil (190°C, 15 s) or oven-baking (208°C, 15 min), packaged (polyethylene bags), and stored at -20°C (7 days). Samples were analyzed for microbial counts before antimicrobial treatment, and after grinding, browning and frozen storage. Data (two replicates, three samples/treatment) were statistically analyzed with independent variables being antimicrobial treatment, sampling point, browning method and their interactions.
Results: Total reductions of inoculated *Salmonella* in control samples were 0.8-1.2 log CFU/g after fryer/oven browning and frozen storage. In comparison, treatment with CAP, CAR, PAA or POL, irrespective of concentration, reduced counts by 3.3->4.5, 4.1->4.7, 1.6->3.9, and 1.1-1.6 log CFU/g, respectively. Treatment with 1.0% CAP (oven-browned) or 0.5% CAR (fryer/oven-browned) reduced *Salmonella* to non-detectable levels (<0.3 log CFU/g) in stored frozen product. Oven-browned samples treated with 0.5% CAP, or 0.3 or 0.5% PAA had lower ($P<0.05$) *Salmonella* counts than corresponding fryer-browned samples.
Significance: These data may be useful for the selection of suitable antimicrobials to reduce the risk of *Salmonella* contamination in surface-browned, uncooked, frozen breaded chicken meat products.

ABSTRACT ACCEPTED FOR POSTER PRESENTATION AT THE ANNUAL MEETING OF THE INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION (JULY 31-AUGUST 3, 2011; MILWAUKEE, WI)

Title: Antimicrobial Treatments for Reduction of *Salmonella* Contamination in Not Ready-To-Eat, Surface-Browned, Frozen, Breaded Chicken Entrees

Authors: Galatios D. Moschonas, Ifigenia Geornaras, Jarret Stopforth, and John N. Sofos

Introduction: Not ready-to-eat, frozen, breaded chicken entrees that are surface-browned to induce a desirable golden-brown color, have been identified as sources of salmonellosis outbreaks when consumed without adequate cooking.
Purpose: The aim of this study was to develop antimicrobial interventions for reduction of *Salmonella* contamination in such products.
Methods: Fresh chicken breast meat portions (5×5×5 cm; 2 kg) were inoculated with *Salmonella* (20 ml; 7-strain mixture; 5 log CFU/g) and mixed with distilled water (control) or with each of seven levels of caprylic acid (CAA; 0.03125 to 1.0%), six levels of carvacrol (CAR; 0.0375 to 0.5%) or four levels of ε-polylysine (POL; 0.125 to 0.5%), applied individually or in combinations of two or three, for a total of 25 treatments. Sodium chloride (1.2%) and sodium tripolyphosphate (0.3%) were added to all treatments (5% total moisture enhancement level), and then ground and formed into 9×5×3 cm portions. Samples were glazed with beaten egg whites, rolled in breadcrumbs, browned by oven-baking (208°C, 15 min), packaged in polyethylene bags, and stored at -20°C for 7 days. Samples were analyzed for microbial counts before antimicrobial treatment, and after grinding, browning and frozen storage. Data (two repetitions, three samples/treatment/repetition) were statistically analyzed with independent variables being antimicrobial treatment, sampling point, and their interactions.
Results: Total reductions of inoculated *Salmonella* in control samples ranged from 0.8 to 1.4 log CFU/g after browning and frozen storage. In comparison, single treatments of CAA, CAR or POL reduced counts by 2.9 to >4.5, 3.4 to >4.4 and 1.4 to 2.3 log CFU/g, respectively, depending on concentration. Levels of 0.0625 to 0.25% CAA, 0.075 to 0.3% CAR or 0.5% POL, applied in combinations of two or three, reduced ($P<0.05$) *Salmonella* counts in stored frozen products by 1.7 to >4.6 log CFU/g. Specifically, combinations of CAA (0.0625 to 0.25%) with CAR (0.075 to 0.3%) reduced *Salmonella* counts by 1.8 to >4.2 log CFU/g. Combinations of 0.0625, 0.125 or 0.25% CAA with 0.5% POL reduced counts by 1.8, 1.7, and 2.6 log CFU/g, respectively, while combinations of 0.075, 0.15 or 0.3% CAR with 0.5% POL reduced counts by 2.0, 3.2, and >4.5 log CFU/g, respectively. Combination of all three antimicrobials reduced ($P<0.05$) *Salmonella* counts by 2.4 to >4.6 log CFU/g, depending on the concentrations tested.
Significance: These data may be used for the selection of suitable antimicrobials and concentrations to reduce *Salmonella* contamination in not ready-to-eat, surface-browned, frozen, breaded chicken entrees, after evaluation of effects in sensory quality.