

Validation of Thermal Processes Against *Salmonella* spp. and the Effects of Matrix on Lethality

Mary L. Bandu, Ph.D.

Director of Technical Services

Research and Chemistry

Chestnut Labs



Current Regulation – Food Safety Modernization Act (FSMA)

- October 25, 2013 – The FDA issued a proposed rule under the FDA Food Safety Modernization Act (FSMA) aimed at improving the safety of food for animals. This proposed regulation would help prevent foodborne illness in both animals and people and is open for public comments for 120 days.
- January 31, 2014 – Comments may be submitted until March 31, 2014.



<http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm372128.htm>
<http://www.fda.gov/Food/NewsEvents/ConstituentUpdates/ucm383490.htm>



FSMA

- **Primary focus is on prevention of food safety incidences**
- **Hazards to food safety should be identified**
 - Microbiological
 - Chemical
 - Physical
 - Radiological
- **Preventative Controls should be implemented**
- **Controls should be validated to ensure hazards will be minimized**

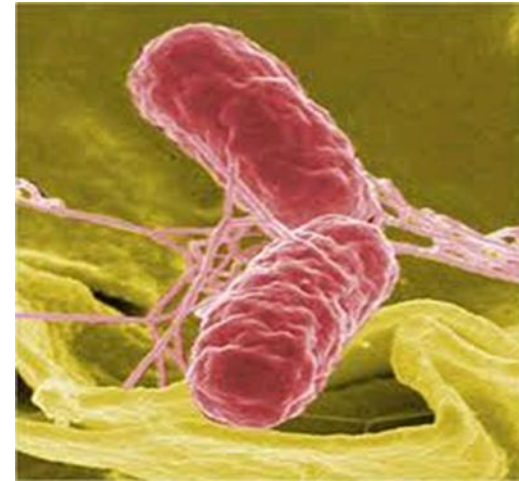


Microbial Hazard for Pet Food – *Salmonella* spp.

Examples of “*Salmonella*-sensitive” ingredients used in low-moisture products*

- Chocolate, chocolate liquor, cocoa powder, chocolate chips, cocoa products
- Nuts/nut products
- Coconuts
- Seeds/seed products
- Grains/grain products (excluding starches)
- Dried egg products
- Fruits/fruit products
- Dairy ingredients and blends
- Spices/herbs (excluding extracts), blended seasonings
- Soy products
- Gums/thickeners (excluding xanthan gum)
- Yeast/yeast extract
- Gelatin
- Dry vegetables
- Enzymes/rennets
- Dry meat or meat byproducts

*This list is not inclusive of all sensitive ingredients.

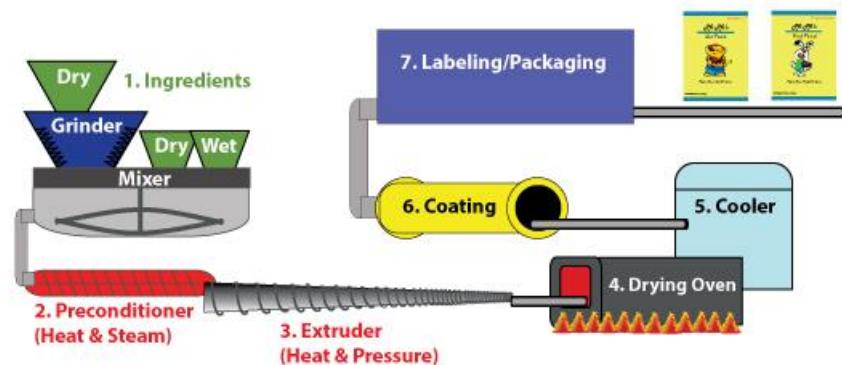


<http://www.gmaonline.org/downloads/technical-guidance-and-tools/SalmonellaControlGuidance.pdf>
<http://www.zimbio.com/Nanotechnology/articles/b5ScwnCzb1C/Rapid+test+for+Salmonella>



Preventative Controls

- Extrusion and baking conditions are lethal to *Salmonella* spp.
 - Heat
 - Steam
 - Pressure
 - Drying



<http://www.petfoodinstitute.org/?page=DryPetFood>

Validation of Preventative Controls

- **Under the proposed rule (507.45), validation of preventative controls is required to ensure control of the hazards identified**
 - Validation through experiments
 - Validation through documentation



Validation - Lab Experiments

- **The matrix of interest is prepared in the lab**
- **The matrix is inoculated with high levels of the pathogen of concern**
- **The matrix is processed using manufacturing conditions**
- **After processing, final organism counts are compared to initial counts to determine the overall \log_{10} reduction of organism**

http://www.fsis.usda.gov/wps/wcm/connect/3b52f9c0-0585-4c0aabf2-b4fc89a9668c/NACMCF_Inoculated_Pack_2009F.pdf?MOD=AJPERES



Validation – In Plant Experiments

- **A suitable non-pathogenic surrogate organism is identified**
- **The matrix of interest is prepared at the plant**
- **The matrix is inoculated with high levels of the surrogate**
- **The matrix is processed in the plant**
- **After processing, final organism counts are compared to initial counts to determine the overall \log_{10} reduction of organism**



Validation - Documentation

- **FSMA states “The scientific and technical information ...may include scientific publications, government documents, predictive mathematical models and other risk-based models, and technical information from equipment manufacturers, trade associations, and other sources.”**
- **In addition, “the conditions used by the (manufacturing) facility are consistent with those described in the supporting literature.”**
 - Temperature
 - Heating Time
 - Ingredient Matrix



Current Investigation – Matrix Effects

- **Pet treat formulation variations**
 - Base formulation with low fat and high moisture
 - Base formulation with low fat and low moisture
 - Base formulation with high fat and low moisture



Experimental Conditions – Dough Preparation

| Pet Treat Recipe | | | |
|---------------------|--------------|----------|---------|
| Ingredient | Low Moisture | High Fat | Base |
| Flour | 2 cups | 2 cups | 2 cups |
| Meal (Porcine) | 2/3 cup | 2/3 cup | 2/3 cup |
| Egg | 2 eggs | 2 eggs | 2 eggs |
| Chicken Broth | | | 1/2 cup |
| Oil | 3/4 cup | 2 Tbsp | 2 Tbsp |
| Milk | | | 1/4 cup |
| High Fat Shortening | | 3/4 cup | |

- **50 grams of dough was formed for each treat**
- **Treats were formed in an oval mold with an approximate dimension of 3 in (length) X 2.65 in (width), and 0.5 in (depth)**

<http://www.dogtreatkitchen.com/dog-biscuit-recipe.html>



Experimental Conditions – *Salmonella* spp.

- **Cocktail**

| | |
|---|----------------|
| Salmonella enterica subsp. Enterica PT-30 | ATCC BAA- 1045 |
| Salmonella enterica subsp. enterica serovar Tennessee | ATCC 10722 |
| Salmonella enterica subsp. enterica serovar Senftenberg | ATCC 8400 |
| Salmonella enterica subsp. enterica serovar Enteritidis | ATCC 13076 |
| Salmonella enterica subsp. enterica serovar Typhimurium | ATCC 14028 |

- **2 mL of cocktail was added to bulk dough preparations**
- **Final inoculum concentration was $\sim 10^7$ CFU/g**



Experimental Conditions – Thermal Processing

- **Pet food was baked in a conventional oven at 220 °F**
- **Samples were pulled from the oven every 10 minutes for 40 minutes**
- **Triplicate samples were evaluated at each testing point**



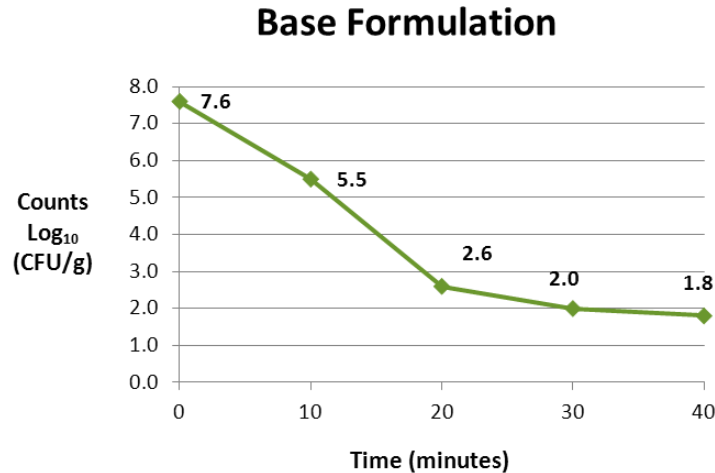
Experimental Conditions – Sample Analysis

- **11 gram samples were diluted 1:10 in BPB**
- **Diluent was hand massaged for approximately 2 minutes**
- **Samples were plated onto Xylose Lysine Deoxycholate Agar (XLD)**
- **Plates were incubated at 35 °C for 24 – 48 hours**



Results – Base Formulation

High Moisture/Low Fat



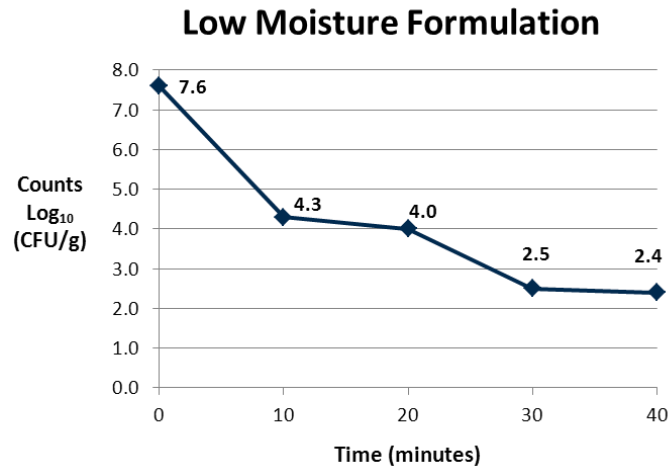
| Ingredient | Base Formulation |
|---------------------|------------------|
| Flour | 2 cups |
| Meal (Porcine) | 1/2 cup |
| Egg | 2 eggs |
| Chicken Broth | 1/4 cup |
| Oil | 2 Tbls |
| Milk | 1/2 cup |
| High Fat Shortening | |

- Thermal processing resulted in a 5.8 log₁₀ reduction in *Salmonella* spp.



Results – Low Moisture Formulation

Low Moisture/Low Fat

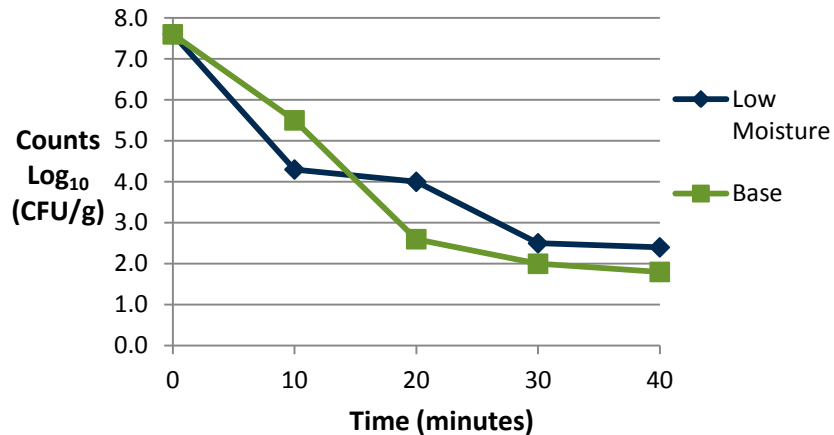


| Ingredient | Low Moisture |
|---------------------|--------------|
| Flour | 2 cups |
| Meal (Porcine) | 1/2 cup |
| Egg | 2 eggs |
| Chicken Broth | |
| Oil | 3/4 cup |
| Milk | |
| High Fat Shortening | |

- Thermal processing resulted in a 5.2 log₁₀ reduction in *Salmonella* spp.



Results - Low Moisture vs. Base Formulation



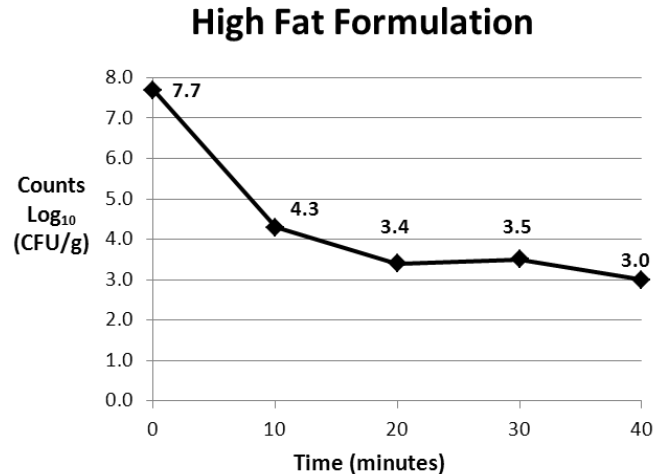
| Ingredient | Low Moisture | Base |
|---------------------|--------------|---------|
| Flour | 2 cups | 2 cups |
| Meal (Porcine) | 1/2 cup | 1/2 cup |
| Egg | 2 eggs | 2 eggs |
| Chicken Broth | | 1/4 cup |
| Oil | 3/4 cup | 2 Tbls |
| Milk | | 1/2 cup |
| High Fat Shortening | | |

- The Low Moisture formulation had higher log₁₀ reduction earlier in the bake cycle (10 minutes)
- The largest difference in lethality occurs at 20 minutes (log₁₀ difference of 1.2)
- The Base Formulation has an approximate 0.5 log₁₀ greater reduction in *Salmonella* spp. after 40 minutes of bake time



Results - High Fat Formulation

Low Moisture/High Fat

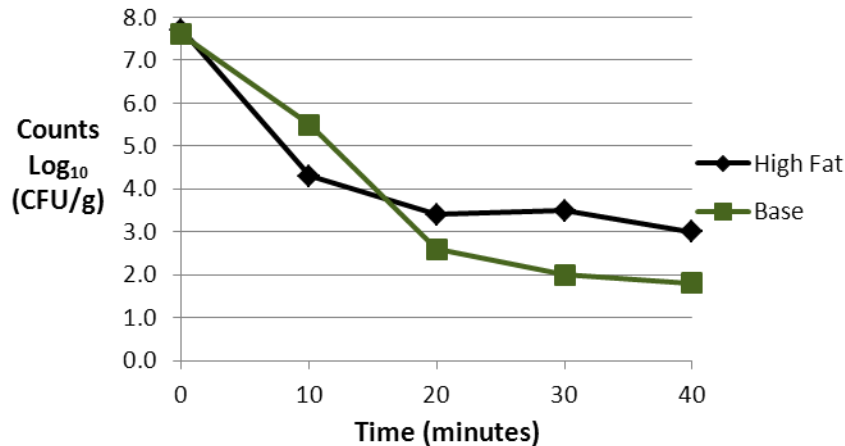


| Ingredient | High Fat |
|---------------------|----------|
| Flour | 2 cups |
| Meal (Porcine) | 1/2 cup |
| Egg | 2 eggs |
| Chicken Broth | |
| Oil | |
| Milk | |
| High Fat Shortening | 3/4 cup |

- Thermal processing for 40 minutes resulted in a 4.7 log₁₀ reduction in *Salmonella* spp.



Results - High Fat vs. Base Formulation



| Ingredient | High Fat | Base |
|---------------------|----------|---------|
| Flour | 2 cups | 2 cups |
| Meal (Porcine) | 1/2 cup | 1/2 cup |
| Egg | 2 eggs | 2 eggs |
| Chicken Broth | | 1/4 cup |
| Oil | | 2 Tbls |
| Milk | | 1/2 cup |
| High Fat Shortening | 3/4 cup | |

- The High Fat formulation had higher log₁₀ reduction earlier in the bake cycle (10 minutes)
- The largest difference in lethality occurs at 30 minutes (log₁₀ difference of 1.5)
- The Base Formulation has an approximate 1.1 log₁₀ greater reduction in *Salmonella* spp. after 40 minutes of bake time



Thermal Resistance

- **Raw materials from vendors may have previous thermal processing**
- **Re-work materials have previous thermal processing**
- **If previous thermal processes are not sufficient, *Salmonella* can survive**
- **Colonies that survive thermal processes are resistant to future thermal processing procedures and may not produce the desired lethality**

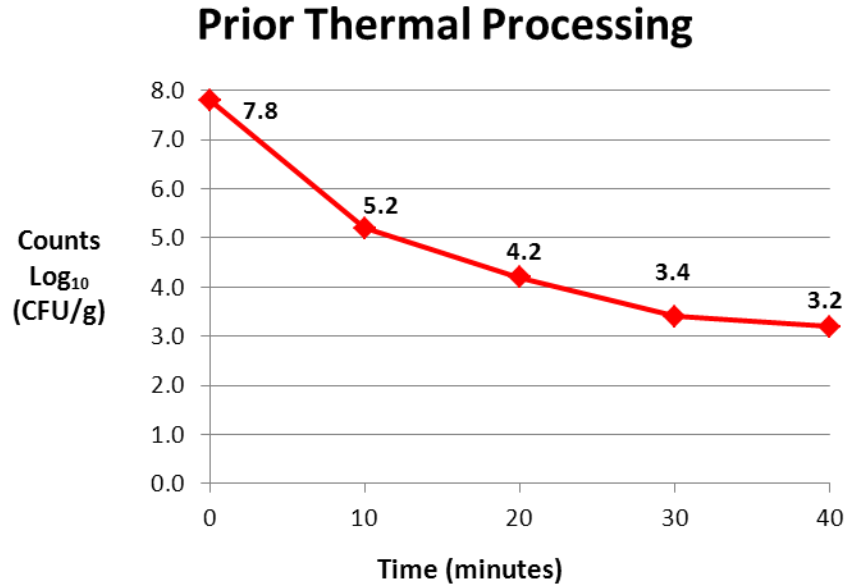


Thermal Resistance Investigation

- **Surviving organisms from the Low Moisture thermal processing experiment were cultivated**
- **Organisms were re-inoculated into the Low Moisture Formulation**
- **The new batch was re-processed under the same experimental conditions**



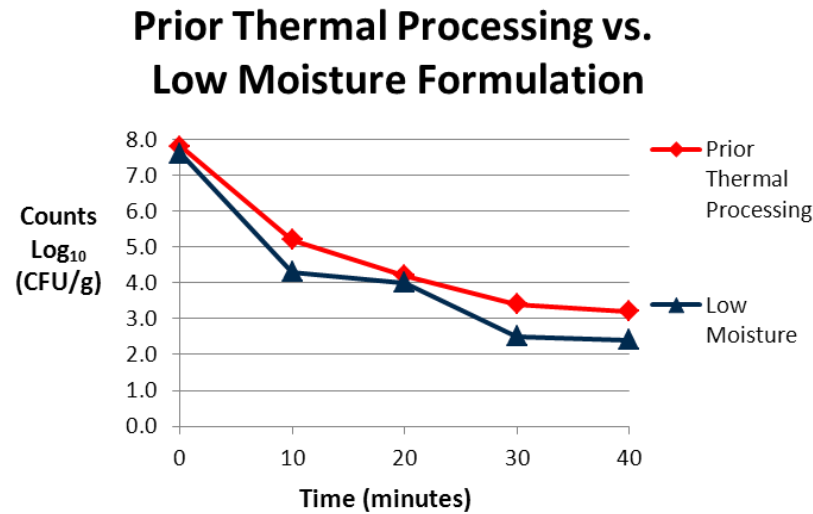
Results – Thermal Resistance



- Thermal processing for 40 minutes resulted in a 4.6 log₁₀ reduction in *Salmonella* spp.



Results - Prior Thermal Processing vs. Low Moisture Formulation

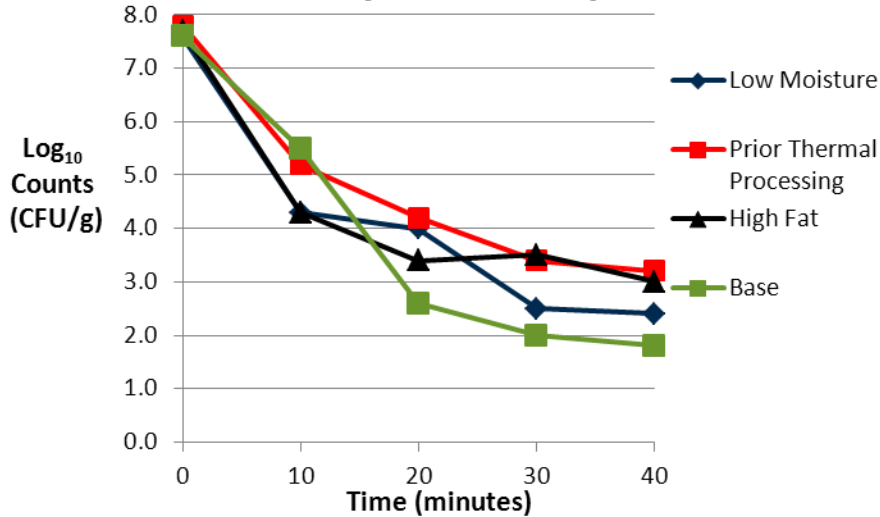


- The Prior Thermal Processing test group had a lower log₁₀ reduction in organism counts throughout the test interval
- The Base Formulation has an approximate 0.8 log₁₀ greater reduction in *Salmonella* spp. after 40 minutes of bake time

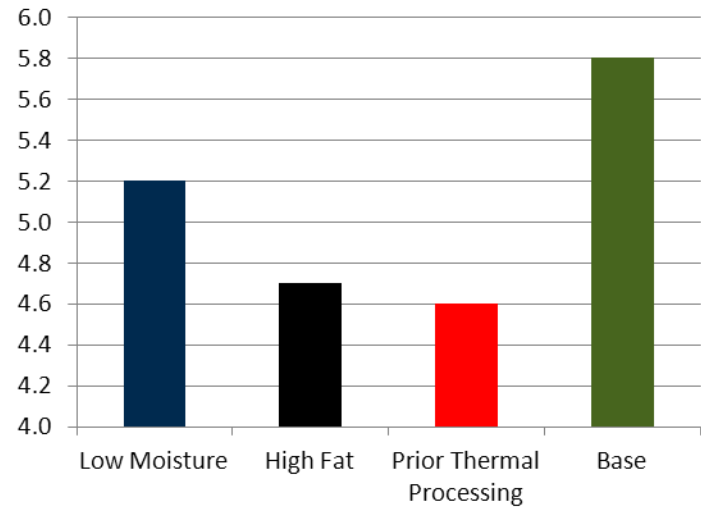


Results – All Test Groups

Log₁₀ Reduction of Organism by Test Group



Final Log₁₀ Reduction by Test Group



| Ingredient | Low Moisture | High Fat | Base |
|---------------------|--------------|----------|---------|
| Flour | 2 cups | 2 cups | 2 cups |
| Meal (Porcine) | 1/2 cup | 1/2 cup | 1/2 cup |
| Egg | 2 eggs | 2 eggs | 2 eggs |
| Chicken Broth | | | 1/4 cup |
| Oil | 3/4 cup | | 2 Tbls |
| Milk | | | 1/2 cup |
| High Fat Shortening | | 3/4 cup | |



Summary

- **The Base Formulation had the highest \log_{10} reduction of *Salmonella* followed by the Low Moisture Test Group, the High Fat Test Group, and lastly, the Prior Thermal Processing Test Group**
- **Moisture in pet food formulations is beneficial in reducing *Salmonella* during thermal processing**
- **Oils and shortenings have an insulating effect which can protect *Salmonella* during thermal processing**
- ***Salmonella* survivors in thermally processed matrices can be resistant to subsequent thermal processes**



Summary

- **As the results indicate, matrix ingredients can effect the outcome of validation experiments**
- **When using documentation (publications, vendor material, regulatory guides) to validate Preventative Controls, follow the FSMA proposed regulation by ensuring:**
 - **“the conditions used by your (manufacturing) facility are consistent with those described in the supporting literature.”**
 - Temperature
 - Heating Time
 - Ingredient Matrix



Thanks for Your Time

- Questions?

