THESIS

LOCATION OF *SALMONELLA* IN POULTRY FAT INTENDED FOR USE IN PET FOOD AND THE INFLUENCE OF FAT'S PHYSICAL CHARACTERISTICS ON *SALMONELLA* PREVALENCE AND GROWTH

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ABSTRACT

LOCATION OF *SALMONELLA* IN POULTRY FAT INTENDED FOR USE IN PET FOOD AND THE INFLUENCE OF FAT'S PHYSICAL CHARACTERISTICS ON *SALMONELLA* PREVALENCE AND GROWTH

This study was conducted to: (i) utilize fluorescently-tagged *Salmonella* to assess distribution of *Salmonella* in a rendered fat matrix; (ii) assess the influence of post-inoculation time and moisture content on distribution of fluorescently-tagged *Salmonella* in rendered poultry fat; and, (iii) evaluate the impact of post-inoculation time and physical parameters (i.e., impurity level and moisture content) on survival of three *Salmonella* serotype strains in rendered poultry fat stored at 25˚C or 45˚C. Three studies, designated as Study I(a), I(b) and II were conducted to address the objectives. In Study I(a), a green fluorescent protein (GFP)-expressing strain of *Salmonella* Typhimurium was used to visually and microbiologically map the organism within warmed (45˚C) poultry fat formulations comprised of a low impurity level (<0.2%) and three moisture contents (low: 0.5%; medium: 2.2%; high: 4.5%). In Study I(b), using the same fat formulations as in Study I(a), survivability of GFP-expressing *Salmonella* was compared in samples that were either stored at 25˚C or 45˚C. In Study II, survivability of three *Salmonella* serotype (Enteritidis, Senftenberg, Typhimurium) strains was compared in fat formulations of two impurity levels (0.5%, 1.0%), three moisture contents (low: 0.5-0.7%; medium: 2.1-3.0%; high: 3.9-4.8%) and two temperatures (25˚C, 45˚C). Surviving populations of *Salmonella* Typhimurium and their location in a rendered fat matrix were achieved for each treatment combination (Study I). For Study I(b) and II, death/survival/growth curves were developed and

comparisons among factors of time, temperature and moisture contents were made. In conclusion, the best option for the rendering industry to control *Salmonella* in poultry fat it is to control multiple factors when storing the final product, more specifically, low impurity poultry fat with low moisture content that is stored at a high temperature (45˚C and above) for a period of time would effectively control *Salmonella* contamination in poultry fat. Preventing recontamination is another crucial point for the rendering facilities, in that matter, GMP is essential, sanitation conditions that will not allow contamination and biofilm formation should be implemented and validated, as appropriate cleaning with scrubbing in holding bins, storage tanks, floors, walls, trucks, everything that have contact with the product.

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CHAPTER I

INTRODUCTION

World population growth is estimated to surpass 10.9 billion people by 2100 *(14).* With that expected population growth in addition to predicted income growth, livestock consumption is expected to increase *(33).* Therefore, livestock production and feed for those animals need to correspondingly increase to satisfy population demand *(23).*

However, for this growth to be possible, the rendering industry is crucial. For each livestock animal, only one half to two thirds on average is considered edible for humans *(22).* The value of the rendering industry in converting this waste (raw materials) into many useful products (byproducts) is tremendous. Around 85% of these byproducts are used in animal feed ingredients, for livestock, poultry, aquaculture, and pets *(22).*

The rendering industry safely transforms raw materials into valuable byproducts. Despite that, the Food Safety Modernization Act (FSMA) has implemented preventative based approaches, which require that the rendering companies validate process-based preventive controls for efficacy in reducing biological hazards *(12).* In this regard, although previous data certainly depicts tremendous reductions in microbial populations during rendering, others *(18, 25)* have noted persistence of pathogens during and following processing. For example, *Salmonella* positive results were found in the finished product (meal), and associated with recontamination, the source were attribute to the environment, such as machines, air, walls, floor *(18)*. Therefore, it is not only essential to control the safety or raw ingredients when obtaining

byproducts; it is also crucial to control cross-contamination during and after processing, including storage of these products.

However, mechanisms by which pathogens are introduced into, and persist within, a complex oil matrix are not well understood. As a result, efforts towards understanding routes of introduction into, distribution within, and influence of physical and environmental parameters on pathogens within rendered animal fats is an imperative component in the development of targeted interventions to assure a safe finished product.

LITERATURE REVIEW

Rendering Industry

Annually, the United States produces and slaughters around 8.8 billion chickens, 29 million cattle and 116 million hogs *(34,35)*. Non-edible byproducts of carcasses that are processed at rendering facilities are around 24.5 billion kilograms annually in the U.S. *(22)*. These byproducts include feathers, skin, hair, horns, feet, heads, blood, organs, muscles, and condemned whole carcasses.

However, raw-materials used in rendering are generally contaminated with microorganisms, some of them pathogenic for humans and animals *(22)*. So, it is incumbent on the rendering industry to handle and process this waste in a safe and integrated system to comply with environmental and disease control requirements. This system consists of collection of raw materials and transport to a rendering facility to be processed. The process involves grinding product into a reduced particle size and transferring it to a cooking vessel (continuous flow or batch configuration). Cooking temperatures range from 115.6˚C to 143.4˚C for 40 to 90 minutes

to solubilize lipids; this step is essential to inactivate bacteria, viruses, parasites and protozoa *(22)*. Subsequently, lipids are separated from protein via centrifugation, and bone and most moisture is extracted.

In short, the rendering process involves application of heat, extraction of moisture and protein, and separation of lipid. By this process, waste is converted into useful products, including: protein meals (meat and bone meal), blood meal and tallow *(23)*.

Rendering is recycling; and it is essential to utilize byproducts to sustainably feed animals, minimize waste, grow livestock production and protect the environment by having this waste treated *(20, 23)*. Without the rendering industry, accumulation of non-edible byproducts would be a hazard to animals, humans and the environment *(22, 24)*.

Food Safety in the Rendering Industry

The rendering industry has expended significant effort to reduce risk of product containing microbiological hazards. The North American rendering industry has recommended the Animal Protein Producers Industry (APPI) Code of Practice *(26)*. The APPI facilitates safety of production and manufacture of byproducts by refining the microbiological and chemical quality of animal proteins and fat. In addition, APPI develops and distributes educational materials (e,g,, the *Salmonella* Reduction Education Program), conducts seminars and assists member companies with weekly verification testing to improve their microbiological safety production *(18, 26)*. The APPI Code of Practice was based on Hazard Analysis and Critical Control Points (HACCP) concept and is voluntary *(22)*.

In North America, there are approximately 300 rendering facilities *(22)*. Each rendering facility is responsible for their own food safety protocol, depending upon their raw materials, process steps, and end products. However, with FSMA, they were just required (09/18/16) to

create and implement current good manufacturing practices (CGMP) following the requirements for the manufacturing, processing, packing and holding by FDA. And by September of 2017, they will be required to implement a Hazard Analysis and Preventive Controls and have a supply chain program (*14*).

Animal Food Regulation

Microbiological safety in animal feed is a concern for animals and humans, as contaminated feed can cause infection and disease in animals and subsequently humans *(13).* In this regard, on January 4 of 2011, the FSMA *(11)* was signed. The FSMA was created to refocus attention from reacting to contamination of food to preventing it. In that manner, all manufacturers and distributors of animal feed are required to develop preventive controls to guarantee safety of their products *(1).*

With FSMA, the Food and Drug Administration (FDA) has authority to implement new rules for preventive controls, develop standards, inspect compliance and respond with mandatory recalls of adulterated products *(11, 23).* Larger companies must comply with Risk-Based Preventive Controls for Food for Animals (*11, 14*) under the FDA FSMA by September 18 of 2017.

Salmonella is a pathogen of concern in animal feed; it is a challenge to eliminate this pathogen. So, *Salmonella* is considered a biological hazard that is reasonably likely to occur in animal feed worldwide *(18).*

FDA considers animal feed and pet food contaminated with *Salmonella* adulterated under the section 402 (a)(1) of the FD&C Act (21 U.S.C. 342 (a)(1)). Accordantly with the Compliacne Policy Guide Sec. 690,800 *Salmonella* in Food for Animals, if the animal feed or ingredients are contaminated with *Salmonella* (except the serotypes: Pullorum, Gallinarum, Enteritidis,

Choleraesuis, Abortuseque, Newport and Dublin), and they are not intended for direct human contact, they are not considered adulterated. Differently for pet food, detection of every serotype of *Salmonella* will be considered adulterated *(18)*.

Pet Food

Pet food is considered adulterated if it contains *Salmonella* spp. According to the American Veterinary Medical Association (AVMA), 36% (43.3 million) of U.S. households own dogs and 30.4% own cats (36.1 million) *(2)*. With increased exposure of households to pet food, it is very important to control foodborne zoonotic diseases that can cause the pet and/or their owners to became ill.

Contaminated pet food can cause illness in humans by direct ingestion, or handling with their hands or utensils, or inappropriate cleaning *(6, 27)*. Also, pets can be afflicted by *Salmonella* (with symptoms or not) via food and, subsequently, become a source for human illness *(6, 13)*. If an infected human is a child, elderly, or immunocompromised, risk that the symptoms can be aggravated and even causes death is elevated.

There are different types of pet food. It can be dry, wet, soft moist; and can be processed by retorting, baking, extrusion *(31)* or be raw. Dry pet food is comprised of grain and vegetable flours, rendered animal protein meal and fat, vegetable fat, and flavorings *(21)*. It is processed by extrusion, a step that uses heat with temperature greater than 92˚C, pressure, and steam to rapidly cook the ingredients and transform them into a dried and coated product *(21, 31)*. Even though dry pet food has around 10% moisture (about 0.5 a_w) and a lethal step (extrusion) for *Salmonella*; in the past decade, outbreaks and recalls still have occurred via such products *(21)*.

In 2012, a total of 49 individuals were infected by *Salmonella* Infantis linked to dry dog food produced by Diamond Pet Foods in the Gaston, SC production facility, of which 38% were

children (under two years old; *5*, *16*). From 24 patients (with available information by Center of Disease Control and Prevention [CDC]), 42% were hospitalized. It was a multistate outbreak in the U.S., and two cases even occurred in Canada *(5)*. Sixteen brands of dry dog and cat food from that plant in South Carolina were recalled *(16)*. Thirty-one dogs were diagnosed as ill from the recalled dry dog food (*16*).

In 2007, 62 people were infected by *Salmonella* Schwarzengrund linked to dry pet food produced by Mars Petcare US at their Pennsylvania facility. Of the total cases, 39% were children (under one-year-old), 32% had bloody diarrhea and 25% were hospitalized. It was a multistate outbreak and, fortunately no deaths occurred *(4)*. From 9 afflicted households with a case, *Salmonella* Schwarzengrund was isolated in 5 of 13 (38%) dog fecal samples and 2 of 22 (9%) dry dog food samples *(3)*.

From the period of October 2015 to September of 2016, seven recalls occurred as a consequence of pet food and pet treats that were contaminated with to *Salmonella (12)*. So, the control of this pathogen is essential. One way to control *Salmonella* in pet food is to control *Salmonella* in pet food ingredients. Most of the proteins, amino acids, and fatty acids in pet foods come from the rendering industry *(31)*. These ingredients are high-quality, affordable and environmental friendly *(23)*. In this regard, it is essential that the rendering industry can guarantee the safety of their products.

Poultry Fat

Annually in the U.S., 5.0 billion kilograms of animal proteins and 4.9 billion kilograms of rendered fats are produced *(22)*. Of rendered fats, half billion Kg produced are poultry fat, and 0.4 billion are fats used in pet food (poultry, beef and pork fats; *22*). Poultry fat is derived only

from poultry offals. Rendered fats used in feed can be a blended tallow, grease, poultry fat, and/or cooking oils *(22)*.

The suggested quality specification for Poultry fat are: minimum (min) of 90% for total fatty acids (TFA;TFA indicates the energy level of the fat); maximum (max) of 15% of free fatty acids (FFA; fat acids are free by hydrolysis, to avoid this hydrolysis, fat should be processed and storage in low moisture levels); max of 1% moisture level (moisture can cause corrosion and rust in the equipment, also when fat is stored, moisture settles making inaccurate samples); max of 0.5% impurities (impurities can be small particles of fiber, bone, hair, hide, and soil. Their presence can clog equipment, such as nozzles or fat handling screens); max of 1%. unsaponifiable levels (it can dilute the energy content); and max of 2% of total moisture, impurity and unsaponifiable combined *(22)*.

Salmonella and Salmonella survival in low water activity (a_w) foods

Salmonella is a rod-shaped bacillus, non-spore-forming, gram-negative bacteria. There are two species of *Salmonella: Salmonella bongori* and *Salmonella enterica*, six subspecies and over 2,500 serotypes *(8, 9)*.

Humans can get *Salmonella* infection (Salmonellosis), by ingesting contaminated food or contaminated water, or from contact with dirty hands after touching an infected animal. Salmonellosis symptoms occur 12 to 72 h after infection and are usually diarrhea, fever and abdominal cramps. Patients are generally fine in 4 to 7 days, but symptoms can progress to an invasive infection, causing hospitalization and/or death *(6, 8)*.

According to CDC, 1.2 million people are estimated to be infected by non-typhoidal *Salmonella* each year in the U.S., and 450 people are estimated to die *(6)*. Compared to other foodborne pathogens, *Salmonella* is responsible for outbreaks and recalls in a variety of foods,

for example almonds, peanut butter, pet food, spices, chocolates, raw poultry, and fresh sprouts *(17, 29)*. This pathogen causes so many outbreaks that it is very important to understand how to control it. *Salmonella* survival depends on numerous factors including temperature, a_w levels, type of food or feed and serotype *(21)*.

Heat treatment has been considered to be the more effective technique to eliminate *Salmonella* in food products *(17)*. Another, and the oldest, form of food preservation is the process of reducing water available to bacteria, thus preventing growth. Most of time, low moisture foods are related to low a_w, but that is not always true. The a_w is water available to the bacteria to grow and ranges from 0 to 1 (pure water). Low a_w foods have values lower than 0.7 *(30)*.

Salmonella can survive for days, weeks, and even years in low a_w foods *(30)*. It is a huge concern that *Salmonella* can survive for long periods of time under such conditions. Therefore, it is not a surprise that most of the outbreaks in the U.S from low a_w foods are related to *Salmonella (30)*.

The best a_w for *Salmonella* growth is 0.99 and optimal temperature is 37 ˚C *(13, 17)*, but as mentioned above, it can survive (not grow) for a long time in low a_w foods. Researchers have revealed that in low a_w foods, other physical factors influence survival of *Salmonella*, such as temperature, presence of fat, acidity, specie, and strain *(21, 31)*. Presence of fat in low a_w foods has been shown to protect *Salmonella* against inactivation. *(29)*. Other studies suggested that *Salmonella* at low a_w can increase their heat resistance *(7)*. All of these factors make it very important to improve understanding of that relationship and the influence on *Salmonella* survival to create or improve interventions to control this foodborne pathogen for each unique food substrate and processing setting.

CHAPTER II

LOCATION OF SALMONELLA IN POULTRY FAT INTENDED FOR USE IN PET FOOD AND THE INFLUENCE OF FAT'S PHYSICAL CHARACTERISTICS ON SALMONELLA PREVALENCE AND GROWTH

MATERIALS AND METHODS

Experimental Design

Three studies, designated as Study I(a), I(b) and II were conducted to address the objectives outlined above. In Study I(a), a green fluorescent protein (GFP)-expressing strain of *Salmonella* Typhimurium was used to visually and microbiologically map the organism within warmed (45˚C) poultry fat formulations comprised of a low impurity level (0.2%) and three moisture contents (low: 0.5%; medium: 2.2%; high: 4.5%). In Study I(b), using the same fat formulations as in Study I(a), survivability of the GFP-expressing *Salmonella* strain was compared in samples that were either stored at 25˚C or 45˚C. In Study II, survivability of three *Salmonella* serotype (Enteritidis, Senftenberg, Typhimurium) strains was compared in fat formulations of two impurity levels (0.5%, 1.0%), three moisture contents (low: 0.5-0.7%; medium: 2.1-3.0%; high: 3.9-4.8%) and two temperatures (25˚C, 45˚C).

Procurement and Preparation of Poultry Fat Formulations

Poultry fat comprised of "low", "medium" and "high" impurity levels was procured. One container per impurity level was received. From each of the batches of poultry fat, three 5-g samples were removed for moisture content analysis. Poultry fat in each container was warmed to 35˚C and thoroughly mixed by vigorous stirring before sample aliquots were removed.

Moisture determination was performed by weighing out 1 ± 0.1 g aliquots, in duplicate, from each 5-g sample and drying the samples in a laboratory convection oven at 60˚C, for 72 h.

Once initial moisture content of each of the impurity levels of poultry fat were known, a Pearson Square calculator was used to determine proportions of poultry fat and added water needed to achieve proposed target moisture levels. Regardless of impurity level, target moisture levels were 0.5% ("low" moisture content), 2.5% ("medium" moisture content) and 4.5% ("high" moisture content). All poultry fat formulations were thoroughly mixed after addition of any added moisture, following which, three 5-g samples were collected for moisture analysis as described above. Additionally, samples were collected for impurity level analysis, performed by Darling Analytical Labs (Butler, KY), and water activity analysis (AquaLab model series 3, Decagon Devices, Pullman, WA).

Salmonella *Strains*

A GFP-expressing strain of *Salmonella* Typhimurium DT104 ATCC 700408/ISSAGFP (*28*) was used in Study I(a) and I(b). Use of this strain allowed for visualization of the location of fluorescing cells, with a UV light source, within the inoculated poultry fat. This strain also was used in Study II, as were *Salmonella* Enteritidis (isolated from the gastrointestinal tract of live broilers) and *Salmonella* Senftenberg 775W ATCC 43845 (a well-documented, heat-resistant strain used in previously in a Fats and Proteins Research Foundation, Inc.-funded study) *(25)* (Table 1).

Strains were available as frozen cultures in the culture collection of the Food Safety/Microbiology laboratory of the Center for Meat Safety & Quality (Department of Animal Sciences, Colorado State University, Fort Collins, CO). Strains were activated by transferring an aliquot of frozen culture into 10 ml tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks,

MD) and incubating at 35˚C (20-24 h). Working cultures were maintained on xylose lysine deoxycholate agar (XLD; Acumedia-Neogen, Lansing, MI).

All these three *Salmonella* serotypes were isolated from rendered products in prior studies *(13,20)*. *Salmonella* Enteritidis and *Salmonella* Typhimurium are associated with foodborne illness *(20)*. And in one of these studies, *Salmonella* Senftenberg were on the top three serovars isolated from the samples *(13)*.

Inoculum Preparation

Two days before each experimental replication, an individual colony of each strain was picked off of each respective XLD agar plate and separately cultured and subcultured (35˚C, 22 ± 0.5 h) in 10 ml of TSB. After incubation, broth cultures were transferred to separate sterile centrifuge tubes and cells were harvested by centrifugation (5,590 × *g*, 15 min, 4˚C; J2-MC, Beckman Coulter). Cell pellets were washed twice with 10 ml of phosphate-buffered saline (PBS, pH 7.4; PBS; Sigma-Aldrich, St. Louis, MO). After the final wash with PBS and decanting of the supernatant, cell pellets were vortexed to generate a thin layer of cells over the bottom of the centrifuge tube. Tubes, with the lids removed, were then placed under a biological safety cabinet for 2 h in an attempt to allow evaporation of some of the remaining moisture associated with the cell pellets. Following the 2-h drying period, cells were resuspended in 10 ml of pre-warmed (35˚C) autoclave-sterilized poultry fat. As shown in our previous work *(25)*, use of pre-warmed poultry fat prevented clumping of cells. The formulation of the poultry fat (i.e., impurity level and moisture content) used for resuspension of cells corresponded with the fat formulation to be inoculated. Inoculum were vortexed and visually inspected for homogeneity before use for inoculation of poultry fat samples. The concentration of each of the prepared inoculants were evaluated by serial tenfold dilution and plating of appropriate dilutions onto

XLD agar and tryptic soy agar (TSA; Acumedia-Neogen). Cell concentrations of inoculum ranged from 8 to 9 log CFU/ml (data not shown).

Study I(a): Visual and Microbiological Mapping of GFP-expressing Salmonella *Typhimurium in Poultry Fat at 45˚C*

This study was initiated with the low, medium and high moisture content formulations of the lowest impurity level of poultry fat received (i.e., 0.2%; Table 2) and two replications (n=2) were performed. For each replication of this study, 15 burettes (50 ml, Eisco, India) constituting one burette per moisture level (low, medium and high) and per sampling time (0, 2, 6, 12 and 24 h) were filled with 50 ml of poultry fat and held in 45˚C (44.0˚C ± 1.0˚C) incubators overnight (around 15 hours) to allow the fat to equilibrate to the test temperature. The equilibration step was necessary since viscosity of fat varies with temperature and a consistent temperature exposure for duration of the trial was imperative. Aliquoted fat within burettes was inoculated the morning following temperature equilibration by gently depositing 50 µl of one of the prepared *Salmonella* Typhimurium inocula to the top of the fat sample. Three different *Salmonella* Typhimurium inocula (each one resuspended in the desire moisture content of the poultry fat) were used to inoculate each of the three moisture content (i.e., low, medium and high) fat formulations; for example, burettes filled with low moisture content poultry fat were inoculated with *Salmonella* Typhimurium cells that had been resuspended in fat of the same composition, etc.

At each of five sampling intervals (0, 2, 6, 12 and 24 h post-inoculation), one burette per moisture content was removed from the incubator and visually inspected for fluorescence using a handheld UV light (365 nm, UVGL-58 Handheld, UVP, Upland, CA). Presence or absence of fluorescence was recorded by photography (Figure 1). Following visual assessment, five

sequential 10 ml samples (designated as samples A through E) were obtained per burette as shown in Figure 2. The time for each 10 ml aliquot (A through E) to be collect was approximately 4 min. Immediately following collection, samples were microbiologically analyzed as outlined below to determine numbers of surviving bacteria over the incubation period, in addition to their location within the poultry fat matrix.

Study I(b): Survival of Salmonella *Typhimurium in Poultry Fat Stored at 25˚C or 45˚C*

In addition to visual and microbiological mapping of GFP-expressing *Salmonella* within the low, medium and high moisture content formulations of the low impurity level of fat, it was thought to be of interest to also compare survivability of this strain within these fat formulations when stored at two incubation temperatures, specifically 25˚C (24.5˚C ± 1.0˚C) and 45˚C (44.0˚C ± 1.0˚C). This study was run concurrently with the mapping study (Study I[a]). In brief, 50 ml of the three moisture content and fat formulations were separately distributed into glass bottles, in duplicate. One set was equilibrated overnight (around 14 hours) at 25˚C and the second set at 45˚C. The next morning, the 50-ml aliquots were inoculated with 50 µl of the *Salmonella* Typhimurium inoculum that corresponded with the moisture content of the fat sample. The target inoculation level was 6-7 log CFU/ml. Inoculated samples were vortexed and sampled at 0, 2, 6, 12 and 24 h post-inoculation by removing a 1-ml aliquot and analyzing it for surviving populations.

Study II: Comparison of Survival of Three Salmonella *Serotype Strains in Poultry Fat with Impurity Levels of 0.5% and 1.0%*

Survivability of three *Salmonella* serotype strains (Enteritidis, Senftenberg, Typhimurium; Table 1) was evaluated in poultry fat containing either 0.5% or 1.0% impurities and formulated to attain three moisture contents (low, medium, high). *Salmonella* survivability at

two temperatures (25˚C, 45˚C) over a 48 h post-inoculation period was evaluated (intervals: 0, 2, 5, 8, 12, 24, 48 h). Three replications (n = 3) were performed on separate days for each impurity level.

The experimental setup was similar to that described for Study I(b). Briefly, on the morning of each replication, 20-ml aliquots of the various fat formulations were separately distributed into 50 ml conical centrifuge tubes. One set of nine tubes (one per strain and moisture content; i.e., 3 strains × 3 moisture contents) was equilibrated for 2.0 ± 0.5 h in an incubator at 25˚C (24.5˚C ± 1.0˚C) and a second set of nine tubes in a 45˚C (45.0˚C ± 1.0˚C) incubator (Figure 3). Following temperature equilibration, each 20-ml sample was inoculated with 20 µl of one of the prepared inocula. There were a total of nine *Salmonella* inocula; one for each *Salmonella* serotype strain, and for each strain, one for each moisture level of fat. Immediately after inoculation, samples were vortexed and 1-ml aliquots were removed and analyzed for 0-h bacterial counts. Inoculated tubes were placed into their respective incubators and sampled again at 2, 5, 8, 12, 24, and 48 h post-inoculation.

Microbiological Analysis

Methodology of Murphy et al. *(25)* was used for microbial analysis of poultry fat samples. This included using warmed (35˚C; in a water bath) 0.1% buffered peptone water (Difco, Becton Dickinson) supplemented with 1% Tween 80 (VWR International, West Chester, PA) (BPW-Tween) for tenfold serial dilution of samples. Furthermore, the first dilution was always performed in a 50 ml conical centrifuge tube and this tube was vortexed for 30 s before removing an aliquot for plating or further dilution. The warm BPW-Tween, the larger surface area of the conical tube compared to that of a regular glass test tube (16×150 mm), and the extended vortexing time resulted in emulsification of the poultry fat, and homogeneous

distribution of bacterial cells within the sample. These procedures mitigated phase separation of the fat samples and ultimately led to an expected tenfold serial dilution of microbial populations *(25)*.

Appropriate dilutions of fat samples were surface-plated, in duplicate, onto a selective agar for *Salmonella* (i.e., XLD agar), as well as a non-selective recovery culture medium (i.e., TSA with 1% sodium pyruvate; TSAp). Bacterial counts obtained from the XLD agar plates were those of the inoculum while those recovered with TSAp included any sub-lethally injured inoculum cells that were able to recover, as well as any background microflora associated with the fat samples. Colonies were manually counted after incubation of XLD agar plates at 35˚C for 48 h and TSAp plates at 25˚C for 72 h. The detection limit of the microbiological analysis was 1 log CFU/ml (10 CFU/ml). Uninoculated poultry fat also was analyzed for counts of any natural microflora associated with raw material product, as well as for presence or absence of any naturally-present *Salmonella* populations.

Statistical Analysis

Colonies plate counts were obtained for each study, transformed in base-ten logs (log CFU/ml) that was used as a response variable.

Study I(a) and I(b) were replicated two times (n=2). For Study I(a) simple means and standard error were obtained by R version 3.2.5 in a summary statistics analysis (Table 3). Study I(b) analysis were separated by culture media (XLD, TSAp), a mixed model was fit with Mixed Procedure of SAS version 9.4. Specifically, moisture content (Low, medium, high), temperature (25˚C, 45˚C) and time (0, 2, 6, 12 and 24 h) and all the interactions were treated as fixed effects. In order to account for repeated observations (over time) on a single bottle, bottle ID was included as a random effect. In addition, the experiment was repeated 2 times, so lab day was

also included as a random effect to account for blocking. Still in Study I(b), dunnett and tukey adjusted pairwise comparisons were considered. Data were presented as least squares means with differences reported using a significance level of $\alpha = 0.05$.

Study II analysis were done separately for each impurity level (0.5, 1%) and culture media (XLD, TSAp) and Mixed Procedure of SAS version 9.4 was used to fit a mixed model. Specifically, moisture (low, medium, high), strain (Enteritidis, Senftenberg, Typhimurium), temperature (25˚C, 45˚C) and time (0, 2, 5, 8, 12, 24, 48) and all interactions were considered fixed effects. As similar to Study (Ib), centrifuge tube ID was included as a random effect to account for repeated observations (over time) on a single centrifuge tube. Study II was replicated 3 times (n=3), and lab day was included as a random effect to account for blocking. Dunnett and tukey adjusted pairwise comparisons were considered. Data are presented as least squares means with differences reported using an α of 0.05.

The moisture contents levels, low, medium and high differ among the impurity levels (Table 2).

RESULTS AND DISCUSSION

Physical Properties of Poultry Fat Formulations

Initial moisture content of the three impurity levels (low: 0.2%; medium: 0.5%; high: 1.0%) of poultry fat, as well as the moisture content of the three moisture-content formulations (low, medium, and high) are shown in Table 2. The range in moisture content, across all impurity levels, following formulation was: Low Moisture Content- 0.5 to 0.7%; Medium Moisture

Content- 2.1 to 3.0%; High Moisture Content- 3.9 to 4.8%. Water activity of each moisture content formulation within each of the three impurity levels is shown in Table 2.

Study I(a): Visual and Microbiological Mapping of GFP-expressing Salmonella Typhimurium in Poultry Fat at 45˚C

Microbial populations ranging from <1.00 to 1.70 log CFU/ml were observed in uninoculated poultry fat samples. Furthermore, no naturally-present *Salmonella* were detected in these samples.

Table 3 shows mean (log CFU/ml) surviving bacterial populations and their location (A-E; Figure 2) within the burette model. At the time of inoculation, sample aliquot A was the furthest from the point of inoculation, while sample aliquot E was at the point of inoculation (Figure 2). At the 0-h sampling time, which occurred within 2 min following inoculation, *Salmonella* Typhimurium was recovered from aliquots C, D and E, irrespective of moisture content, indicating rapid migration of cells through the fat matrix. However, differences were noted in concentration levels of the organism at each of these locations among the fat formulations (Table 3). For the low moisture-content formulation, no surviving *Salmonella* Typhimurium (<1.00 log CFU/ml) were detected after the 0 h sampling time. For medium and high moisture content samples, *Salmonella* were recovered at the 2 h sampling interval for all aliquots (A through E) plated on the non-selective agar (TSAp). In comparison, corresponding *Salmonella* counts recovered on selective agar (XLD) were 2.04 log CFU/ml on just aliquot A (medium moisture-content formulation), and 2.45 to 4.00 log CFU/ml (high moisture-content formulation) lower than those recovered with TSAp (Table 3). Differences in *Salmonella* Typhimurium populations recovered with the non-selective and selective agars suggested presence of sub-lethally injured cells within the fat matrix; these cells were not able to recover

and grow on selective agar, but were able to do so on non-selective agar. Regardless, no surviving *Salmonella* Typhimurium were recovered using either culture medium (TSAp or XLD) from any aliquots of fat formulations sampled at 6, 12 and 24 h post-inoculation, the only exception was aliquot C of the high moisture-content product sampled at 6 h and plated on TSAp (Table 3).

Burettes were photographed after inoculation (Figure 1) and after sampling was completed (Figure 3). Post inoculation, fluorescence from the inoculum was just observed on top of poultry fat in the burette (Figure 1). After sampling, fluorescence from tagged *Salmonella* Typhimurium was observed on the sides of the burettes (Figure 3). Even though florescent protein does not participle in the cell metabolism, therefore nonviable cells can also fluorescence (*15*), the fluorescence on the sides of the burettes suggested that biofilm can be formed, or *Salmonella* persisted, in storage vessels—which may have resulted in cross-contamination or reintroduction of pathogens in a true processing system.

Visually differences between moisture contents were just observed at 0h (Figure 4), where low moisture content had more florescence observed comparing with the medium and high moisture. Visually differences between time were just observed at 0h comparing with 2,6,12 and 24h (Figure 5), at 0h more florescence was observed, suggesting that the force to move the inoculum (not enough time to naturally move) by the opening of the valve for sampling, resulted in more *Salmonella* sticking to the sides.

Study I(b): Survival of Salmonella *Typhimurium in Poultry Fat Stored at 25˚C or 45˚C*

Figures 6 and 7 show death/survival curves for *Salmonella* Typhimurium in fat formulations comprised of the low impurity level (0.2%) and three moisture contents (low: 0.5%; medium: 2.2%; high: 4.5%), stored at 25˚C (Figure 6) and 45˚C (Figure 7), for up to 24 h.

Inoculated populations of *Salmonella* Typhimurium (5.49 to 5.57 log CFU/ml) in the low moisture fat formulation were reduced to non-detectable levels (<1.00 log CFU/ml) by 6 h (XLD agar counts) and 12 h (TSAp counts) of incubation at 25˚C (Figure 6). On the other hand, high numbers of surviving *Salmonella* Typhimurium (4 to 6 log CFU/ml) were still culturable in medium and high moisture-content fat samples at the end (24 h) of the 25˚C incubation period. Increasing the incubation temperature from 25˚C to 45˚C resulted in quicker lethality of *Salmonella* in low moisture poultry fat (Figure 7). Furthermore, no recoverable *Salmonella*, at or beyond 6 h incubation, were obtained in medium and high moisture-content fat samples (Figure 7). Regardless of incubation temperature, no recoverable *Salmonella* were obtained from the low moisture poultry fat beyond 6 h of incubation. In contrast, *Salmonella* were recovered from medium and low moisture samples incubated at 25˚C for 6 h and longer (Figure 7).

Study II: Survival of Three Salmonella *Serotype Strains in Poultry Fat with a 0.5% Impurity Level*

No *Salmonella* were recovered from uninoculated poultry fat samples. On TSAp, generic microflora recovered from uninoculated samples ranged from <1.00 to 1.00 log CFU/ml. Initial (0 h) populations of *Salmonella*, for each formulation immediately following inoculation, are presented in Table 4.

Death/survival curves for the three *Salmonella* serotype strains in formulations representing the medium (0.5%) impurity level and three moisture contents (low: 0.5%; medium: 2.1%; high: 3.9%) stored at 25˚C or 45˚C (48 h) are shown in Figures 8 and 9, respectively.

Incubation temperature had a notable effect on survival of all three strains of *Salmonella*; higher numbers of survivors were obtained at 25˚C than at 45˚C (Figures 8 and 9). Even though 45˚C is not considered a high enough temperature for thermal inactivation of *Salmonella* (in fact,

growth of *Salmonella* at 45˚C has been reported under certain conditions; *19*), results of our study showed that, with time and in combination with other substrate-associated factors (moisture, impurity, water activity), incubation of poultry fat at 45˚C resulted in reductions of inoculated *Salmonella* populations.

Furthermore, as reported previously for low impurity (0.2%) fat products, moisture content also had an effect on *Salmonella* survival. Populations of all three strains in the low moisture poultry fat incubated at 25˚C steadily declined over the 48 h period (Figure 8). Also, as with low impurity poultry fat, low moisture content product had a low a_w (Table 2); therefore, reduced survival of *Salmonella* was expected compared to that obtained for medium and high moisture-content formulations ($a_w > 0.8$; more water available to bacteria to survive). Previous studies have reported that *Salmonella* is able to survive for long periods of time in low a_w foods *(7)*. For example, Uesugi et al. study *(32)* showed no reductions over a 550-day storage period at -20˚C and 4˚C of *Salmonella* Enteritidis phage type 30 on almond kernels. The same *Salmonella* behavior was not observed in our study, solidifying that *Salmonella* survival is not just influenced by one factor, but instead by an interaction of factors.

In contrast to low moisture content, *Salmonella* populations of >6 log CFU/ml were obtained even after 48 h at 25˚C in the corresponding medium and high moisture-content formulations (Figure 8). Within each incubation temperature, sporadic differences ($P < 0.05$) in survival between the three *Salmonella* strains were noted (Figure 8 and 9).

Study II: Survival of Three Salmonella *Serotype Strains in Poultry Fat with a 1.0% Impurity Level*

No *Salmonella* were recovered from any of the uninoculated 1.0% impurity fat samples. Generic microflora recovered with TSAp ranged from <1.00 to 3.61 log CFU/ml. Inoculated levels of the three *Salmonella* serotypes, for each tested fat formulation, are presented in Table 5.

Figures 10 and 11 show the death/survival/growth curves of the three *Salmonella* serotype strains in poultry fat representing high (1.0%) impurity and three moisture contents (low: 0.7%; medium: 3.0%; high: 4.8%), stored at 25˚C (Figure 10) and 45˚C (Figure 11) for up to 48 h.

When fat formulations were incubated at 25˚C, *Salmonella* levels in the low moisture fat steadily declined over time, whereas those in the medium moisture content product remained relatively unchanged throughout incubation (Figure 10). In contrast, growth ($P < 0.05$) of *Salmonella* occurred in high moisture fat incubated at 25˚C (Figure 10). Specifically, *Salmonella* populations, regardless of strain, increased by up to 1 log CFU/ml (TSAp: 0.73-1.04 log CFU/ml; XLD: 0.97-1.07 log CFU/ml) (Figure 10). Likely reasons for the observed growth were a combination of relatively high water activity (0.918 ± 0.023; Table 2), high moisture content (4.8 ± 0.31%; Table 2) and high impurity level (1.0%) of the formulation. As noted for the 0.5% impurity level, temperature had an effect on survival of *Salmonella* strains in the 1.0% impurity formulations. But conversely to counts from the 0.5% impurity level, at 45˚C, greater numbers of survivors for a longer period of time were documented for medium and high moisture contents (Figure 10), indicating that food substrate, in this case impurity level, influences survival *(21).* In addition, in the high moisture level fat stored at 45˚C, temperature was not as effective at reducing populations of the three *Salmonella* strains as was temperature for the other moisture contents (low and medium), ending at 48h with around 4 log CFU/ml concentration all three strains (Figure 11).

These data suggested that control of moisture content, temperature, impurity level and water activity is very important for controlling survival of *Salmonella* spp. in poultry fat at 25 and 45˚C. Based on our experimental design, statistical comparison of data could not be performed among the three impurity levels; however, trends indicated that lower impurity levels and lower moisture contents were better at controlling survival of *Salmonella*. Storage of poultry fat with medium or high moisture content at 25˚C allowed survival of large populations of *Salmonella*, and even permitted growth of the pathogen when a high impurity level, high moisture content and high water activity were available.

Industry application

Based on this work, the best option for the industry to control *Salmonella* in poultry fat it is to control multiple factors when storing the final product, more specifically, low impurity poultry fat with low moisture content that is stored at a high temperature (45˚C and above) for a period of time would effectively control *Salmonella* contamination in poultry fat.

Preventing recontamination is another crucial point for the rendering facilities, in that matter, GMP is essential, sanitation conditions that will not allow contamination and biofilm formation should be implemented and validated, as appropriate cleaning with scrubbing in holding bins, storage tanks, floors, walls, trucks, everything that have contact with the product.

While this study yielded valuable information related to the influence of physical parameters on *Salmonella* survivability in poultry fat, more research is necessary to better understand why *Salmonella* spp. survivability in poultry fat differs among impurity levels. Additionally, although limited in scope, the burette portion of this study allowed visual observation of fluorescently-tagged *Salmonella* Typhimurium on the sides of burettes (Figure 4).

Additional research with industry-representative vessels would be useful in determining this whether or not this occurs in the commercial setting.

Table 1. Strains of *Salmonella* spp. used in the study.

***Salmonella* Serotype**	**Strain ID**	**Source**
Salmonella Enteritidis	FFSRU SE NN	Dr. Thomas Edrington (USDA-ARS-SPA, Food and Feed Safety Research Unit, College Station, TX)
Salmonella Senftenberg 775W	ATCC 43845	--
Salmonella Typhimurium DT104	ATCC 700408/ISSAGFP	Noah et al. (2005)

Table 2. Initial moisture content (%; mean ± SD), ending moisture content after addition of moisture (%; mean ± SD) and their water activity (mean ± SD) for each impurity level of poultry fat.

Impurity level (%)	**Initial Moisture Content**	**Low Moisture**	**Water Activity**	**Medium Moisture**	**Water Activity**	**High Moisture**	**Water Activity**
0.2 ± 0.0	0.4 ± 0.1	0.5 ± 0.2	0.461 ± 0.010	2.2 ± 0.9	0.905 ± 0.003	4.5 ± 0.1	0.923 ± 0.003
0.5 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.571 ± 0.012	2.1 ± 0.5	0.842 ± 0.010	3.9 ± 0.5	0.910 ± 0.005
1.0 ± 0.0	0.0 ± 0.1	0.7 ± 0.2	0.554 ± 0.005	3.0 ± 0.2	0.837 ± 0.025	4.8 ± 0.3	0.918 ± 0.023

SD: standard deviation.

Table 3 Effect of time and moisture content (low: 0.5%; medium: 2.2%; high: 4.5%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 0.2% impurity content, incubated at 45˚C for up to 24 h.

Medium	Time (h)	Sample	Low moisture		Medium moisture		High moisture	
			Mean	SE	Mean	SE	Mean	SE
TSAp	0	A	<1.00	0.00	<1.00	0.00	<1.00	0.00
		B	<1.00	0.00	<1.00	0.00	<1.00	0.00
		C	2.75	0.60	4.74	1.20	5.34	0.05
		D	4.86	0.39	4.69	0.03	6.70	0.34
		E	5.96	0.09	6.63	0.48	7.11	0.14
	2	A	<1.00	0.00	3.97	0.62	5.18	0.29
		B	<1.00	0.00	1.35	0.35	4.18	0.44
		C	<1.00	0.00	3.67	1.05	4.19	1.10
		D	<1.00	0.00	2.83	1.83	3.82	1.62
		E	<1.00	0.00	2.66	1.66	4.29	1.27
	6	A	<1.00	0.00	<1.00	0.00	<1.00	0.00
		B	<1.00	0.00	<1.00	0.00	<1.00	0.00
		C	<1.00	0.00	<1.00	0.00	1.00	0.00
		D	<1.00	0.00	<1.00	0.00	<1.00	0.00
		E	<1.00	0.00	<1.00	0.00	<1.00	0.00
	12	A	<1.00	0.00	<1.00	0.00	<1.00	0.00
		B	<1.00	0.00	<1.00	0.00	<1.00	0.00
		C	<1.00	0.00	<1.00	0.00	<1.00	0.00
		D	<1.00	0.00	<1.00	0.00	<1.00	0.00
		E	<1.00	0.00	<1.00	0.00	<1.00	0.00
	24	A	<1.00	0.00	<1.00	0.00	<1.00	0.00
		B	<1.00	0.00	<1.00	0.00	<1.00	0.00
		C	<1.00	0.00	<1.00	0.00	<1.00	0.00
		D	<1.00	0.00	<1.00	0.00	<1.00	0.00
		E	<1.00	0.00	<1.00	0.00	<1.00	0.00
XLD	0	A	<1.00	0.00	<1.00	0.00	<1.00	0.00
		B	<1.00	0.00	<1.00	0.00	<1.00	0.00
		C	2.16	1.16	4.59	0.99	5.24	0.38

		D	4.61	0.19	5.40	0.86	6.57	0.37
		E	5.12	0.59	6.77	0.66	7.04	0.09
	2	A	<1.00	0.00	2.04	0.73	4.00	0.46
		B	<1.00	0.00	<1.00	0.00	2.75	0.54
		C	<1.00	0.00	<1.00	0.00	2.46	1.46
		D	<1.00	0.00	<1.00	0.00	2.45	1.45
		E	<1.00	0.00	<1.00	0.00	2.51	1.51
	6	A	<1.00	0.00	<1.00	0.00	<1.00	0.00
		B	<1.00	0.00	<1.00	0.00	<1.00	0.00
		C	<1.00	0.00	<1.00	0.00	<1.00	0.00
		D	<1.00	0.00	<1.00	0.00	<1.00	0.00
		E	<1.00	0.00	<1.00	0.00	<1.00	0.00
	12	A	<1.00	0.00	<1.00	0.00	<1.00	0.00
		B	<1.00	0.00	<1.00	0.00	<1.00	0.00
		C	<1.00	0.00	<1.00	0.00	<1.00	0.00
		D	<1.00	0.00	<1.00	0.00	<1.00	0.00
		E	<1.00	0.00	<1.00	0.00	<1.00	0.00
	24	A	<1.00	0.00	<1.00	0.00	<1.00	0.00
		B	<1.00	0.00	<1.00	0.00	<1.00	0.00
		C	<1.00	0.00	<1.00	0.00	<1.00	0.00
		D	<1.00	0.00	<1.00	0.00	<1.00	0.00
		E	<1.00	0.00	<1.00	0.00	<1.00	0.00

SE: standard error.
Mean: Simple mean.
Detection limit = 1.00 log CFU/ml

Table 4. Initial (inoculated) populations (log CFU/ml; mean ± SD) of *Salmonella* spp. strains immediately following inoculation (0 h) as recovered on XLD agar.

			Moisture Content		
Impurity (%)	**Temperature (˚C)**	***Salmonella* Serotype**	**Low**	**Medium**	**High**
0.5	25	*Salmonella* Enteritidis	6.37 ± 0.30	6.63 ± 0.18	6.94 ± 0.09
		Salmonella Senftenberg	5.73 ± 0.31	6.64 ± 0.07	6.59 ± 0.12
		Salmonella Typhimurium	6.74 ± 0.25	6.97 ± 0.09	6.98 ± 0.07
0.5	45	*Salmonella* Enteritidis	5.68 ± 0.47	6.75 ± 0.05	6.82 ± 0.20
		Salmonella Senftenberg	5.08 ± 0.60	6.52 ± 0.08	6.78 ± 0.08
		Salmonella Typhimurium	6.24 ± 0.24	7.03 ± 0.07	7.00 ± 0.05
1.0	25	*Salmonella* Enteritidis	5.72 ± 0.26	6.89 ± 0.07	6.88 ± 0.06
		Salmonella Senftenberg	5.50 ± 0.27	6.66 ± 0.16	6.67 ± 0.11
		Salmonella Typhimurium	6.23 ± 0.18	7.03 ± 0.19	6.98 ± 0.24
1.0	45	*Salmonella* Enteritidis	4.59 ± 0.46	6.75 ± 0.11	6.86 ± 0.16
		Salmonella Senftenberg	4.28 ± 1.00	6.58 ± 0.16	6.64 ± 0.25
		Salmonella Typhimurium	5.48 ± 0.29	7.02 ± 0.21	7.17 ± 0.15

SD: standard deviation.

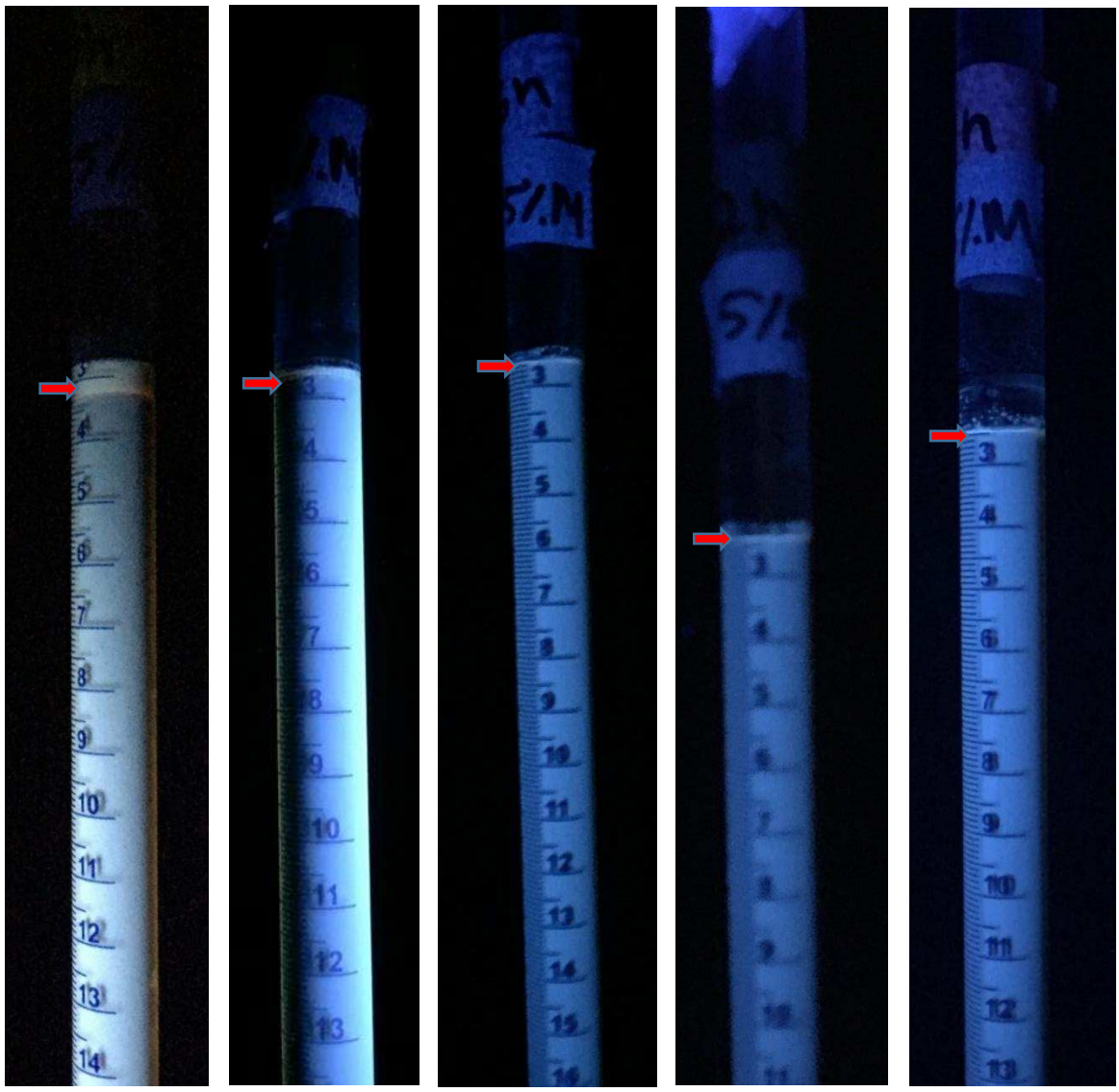

Figure 1. Photographs of the top of the burettes with 50ml of poultry fat after inoculation at 0, 2, 6, 12 and 24h (left to right). Fluorescence was noted at the point of inoculation (i.e., approximately at the 3.5 cm marking of the burette, red arrow).

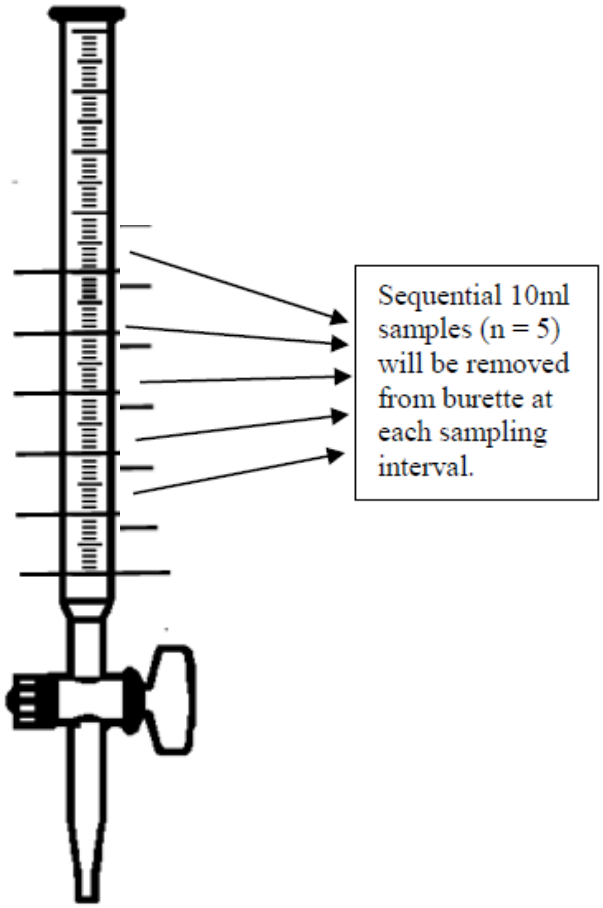

Figure 2. Microbiological sampling schematic for poultry fat at five sampling intervals.

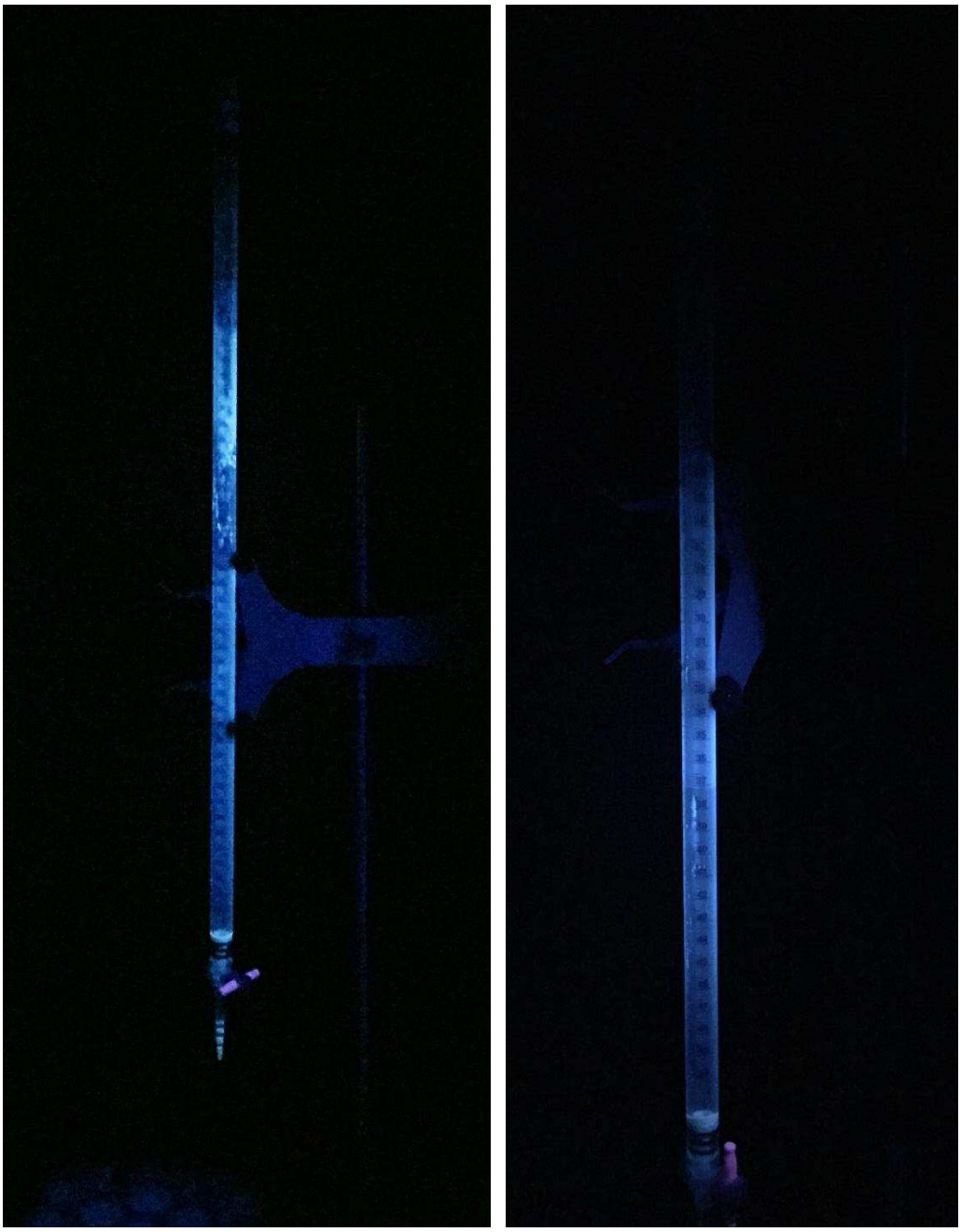

Figure 3. Photographs of empty burettes after sampling was completed. Fluorescence was noted along the sides of the empty burettes.

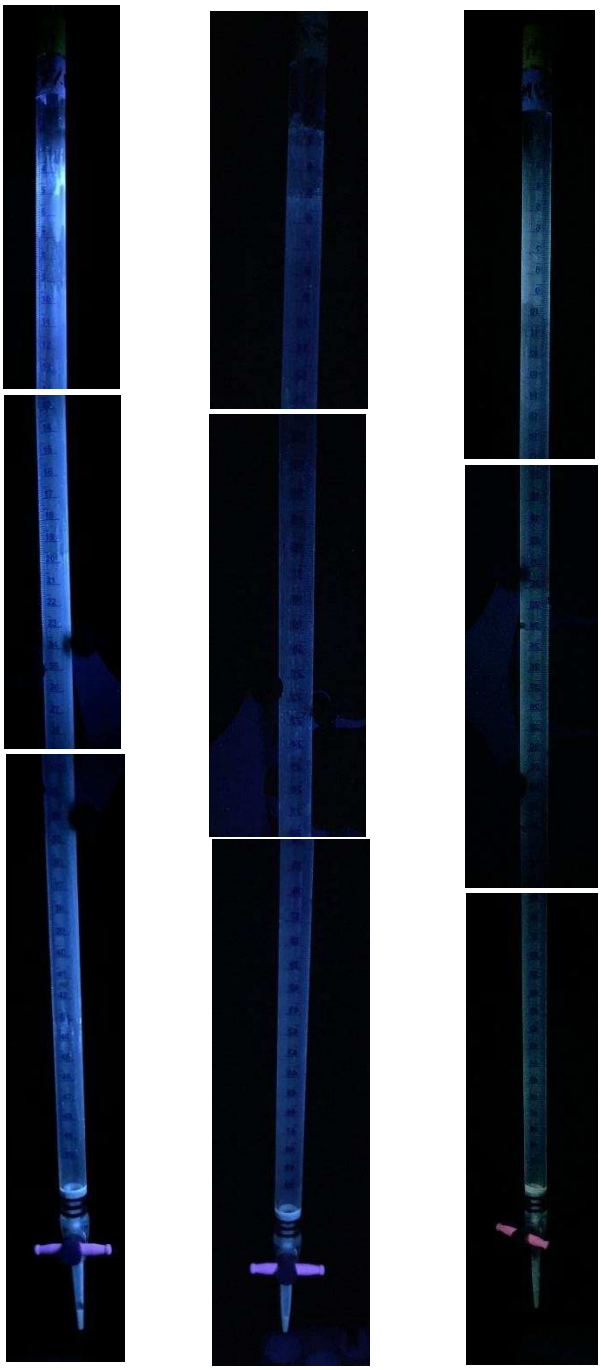

Figure 4. Photographs of the top, medium and bottom of empty burettes after sampling was completed at 0h for low moisture, medium moisture and high moisture (left to right).

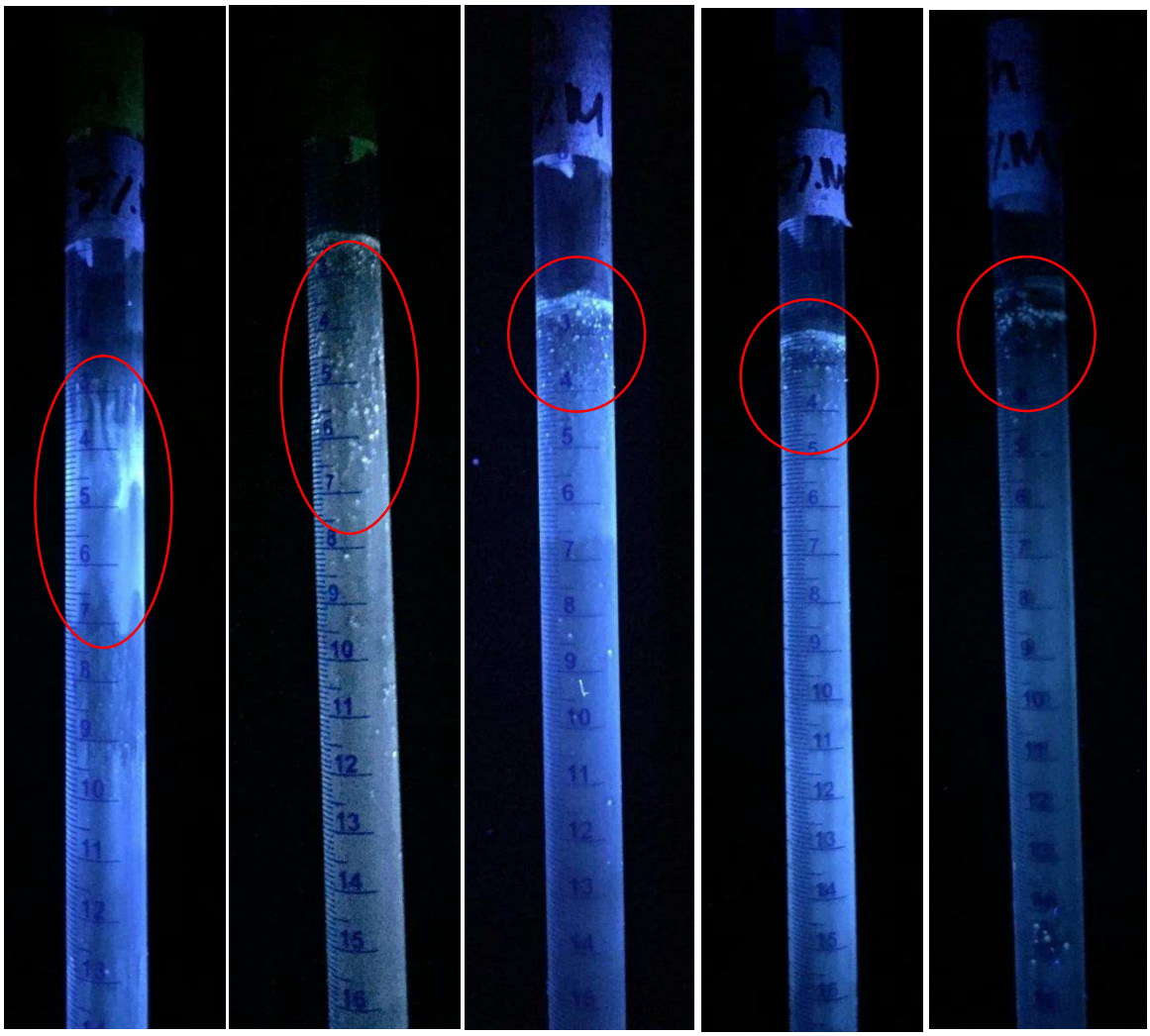

Figure 5. Photographs of the top of empty burettes after sampling was done at 0, 2, 6, 12 and 24h (left to right). Red circles feature the area with more fluorescence.

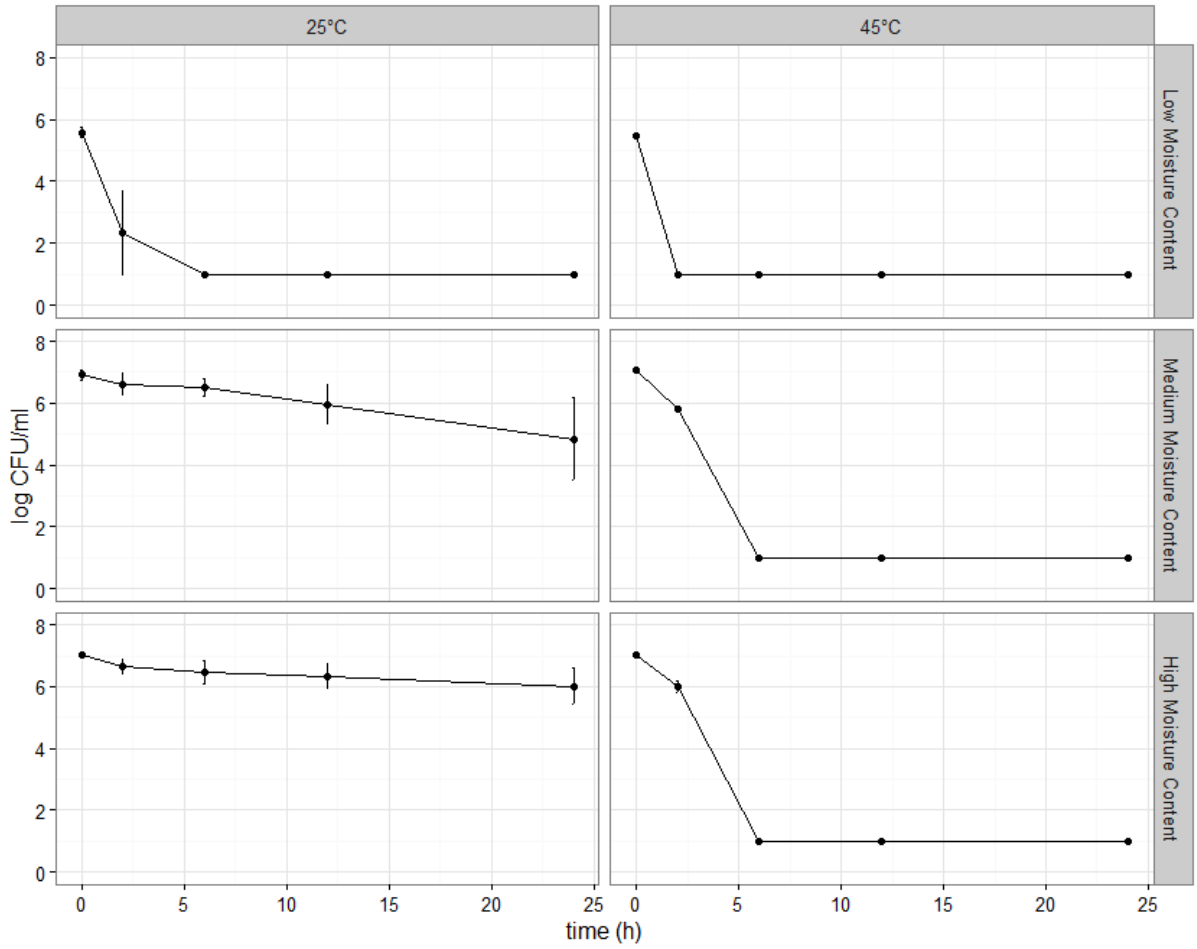

Figure 6. The survival (log CFU/ml) in TSAp of *Salmonella* Typhimurium in 0.2% impurity level poultry fat incubated at 25˚C or 45˚C for 24 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.5 ± 0.2%; medium: 2.2 ± 0.9%; high: 4.5 ± 0.1%). Plot shows standard error bar.

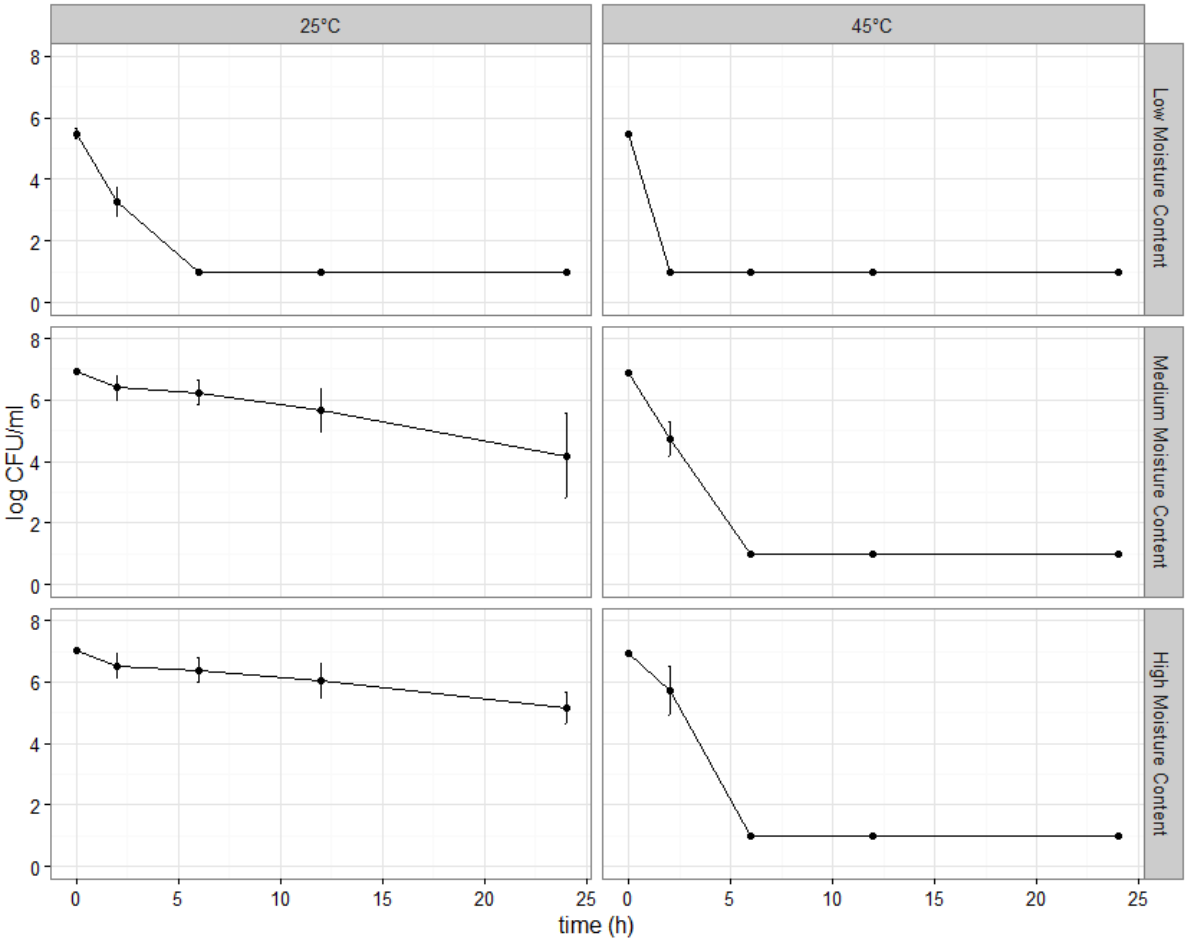

Figure 7. The survival (log CFU/ml) in XLD of *Salmonella* Typhimurium in 0.2% impurity level poultry fat incubated at 25˚C or 45˚C for 24 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.5 ± 0.2%; medium: 2.2 ± 0.9%; high: 4.5 ± 0.1%). Plot shows standard error bar.

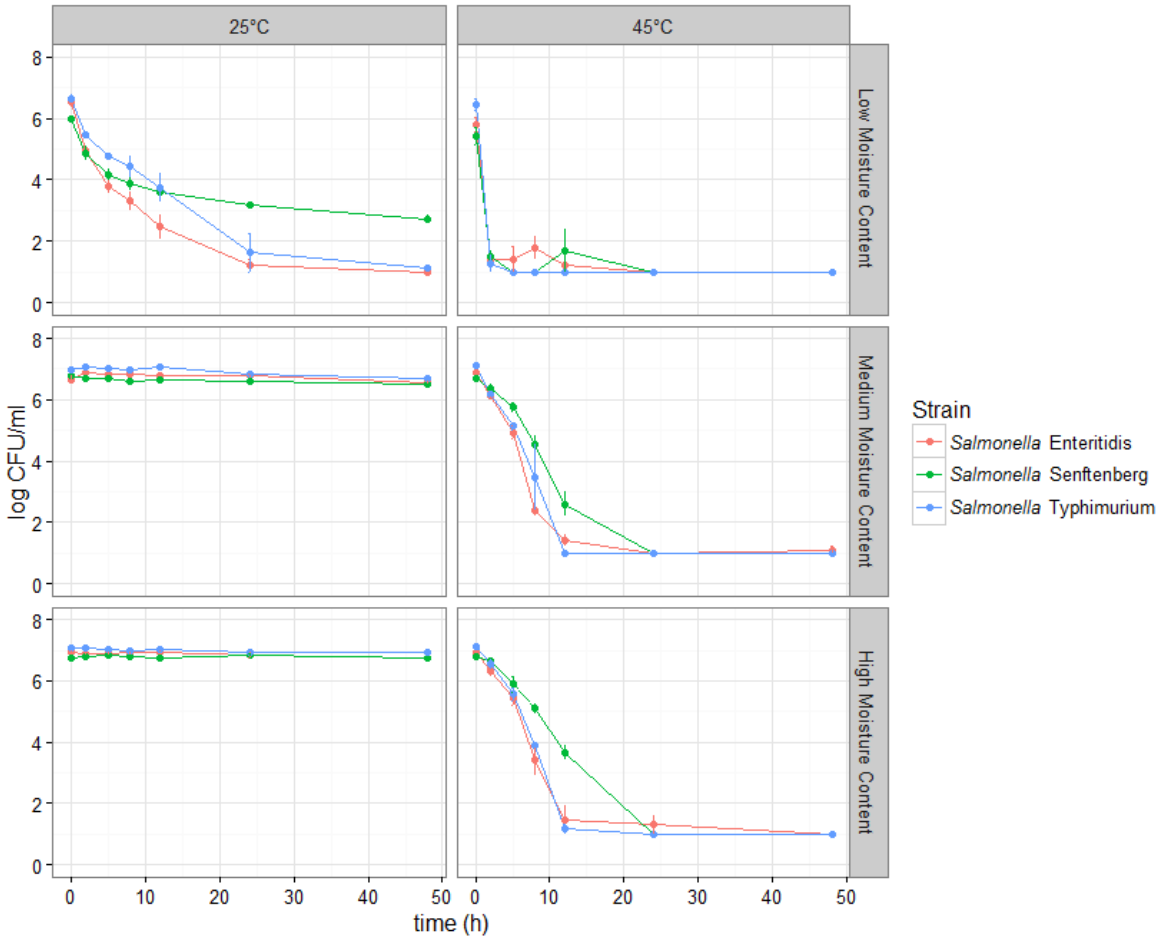

Figure 8. The survival (log CFU/ml) in TSAp of *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium in 0.5% impurity level poultry fat incubated at 25˚C or 45˚C for 48 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.5 ± 0.1%; medium: 2.1 ± 0.5%; high: 3.9 ± 0.5%). Plot shows standard error bar.

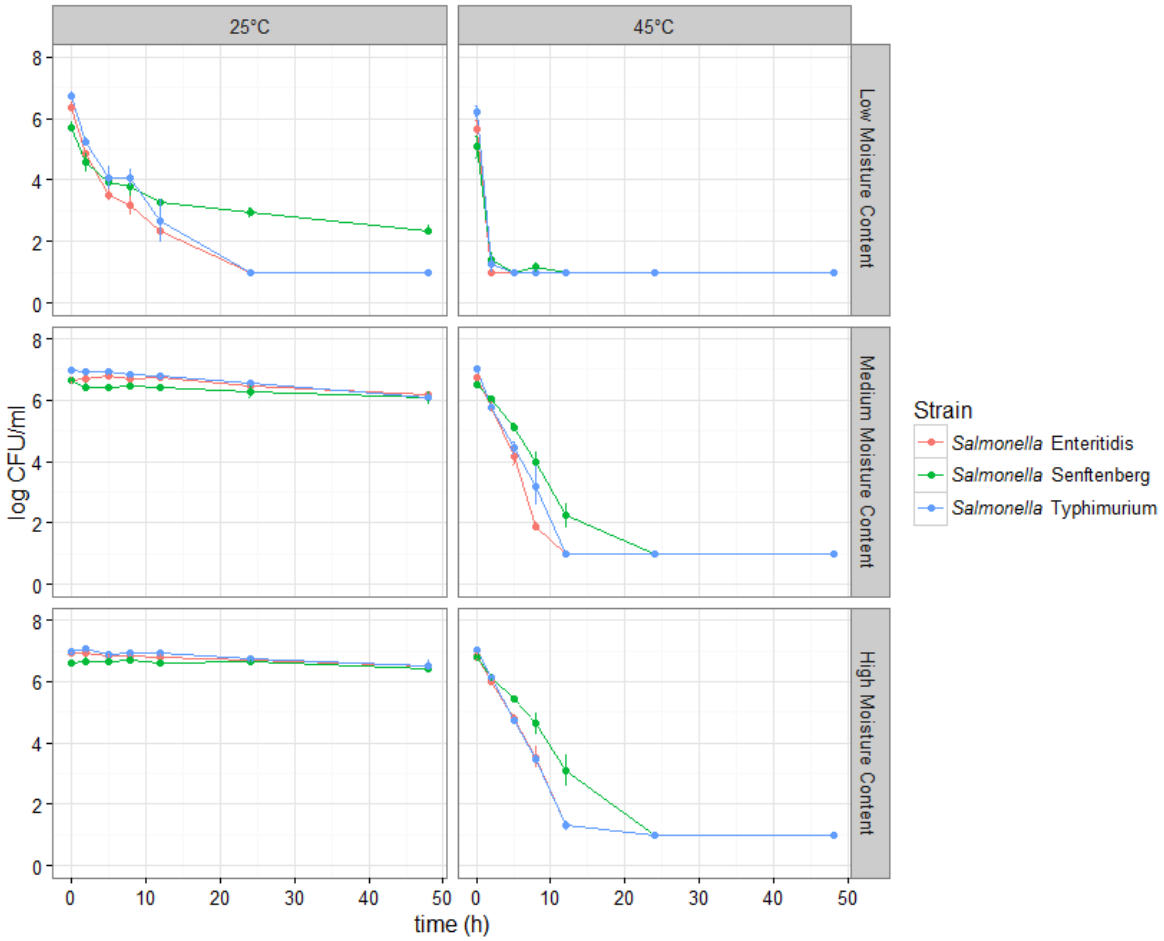

Figure 9. The survival (log CFU/ml) in XLD of *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium in 0.5% impurity level poultry fat incubated at 25˚C or 45˚C for 48 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.5 ± 0.1%; medium: 2.1 ± 0.5%; high: 3.9 ± 0.5%). Plot shows standard error bar.

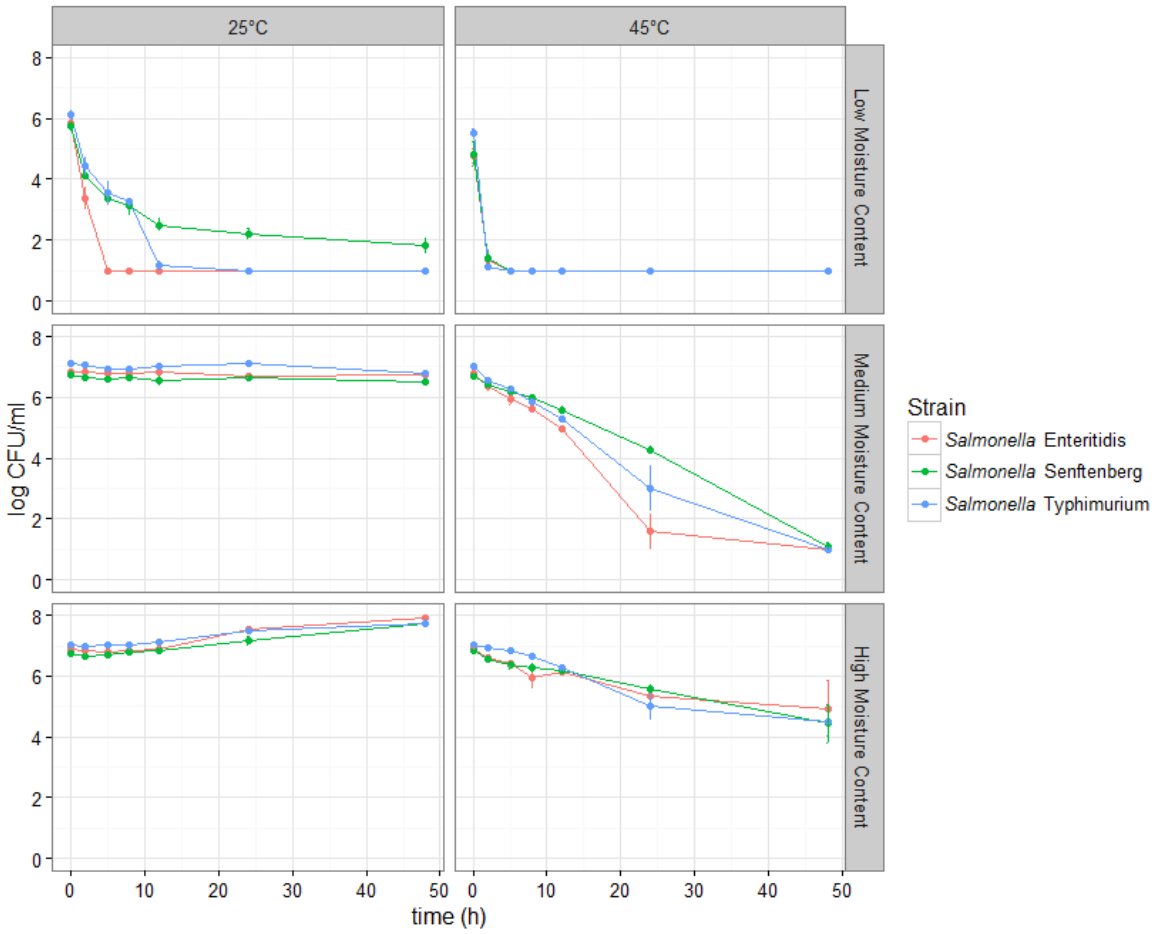

Figure 10. The survival (log CFU/ml) in TSAp of *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium in 1.0% impurity level poultry fat incubated at 25˚C or 45˚C for 48 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.7 ± 0.2%; medium: 3.0 ± 0.2%; high: 4.8% ± 0.3%). Plot shows standard error bar.

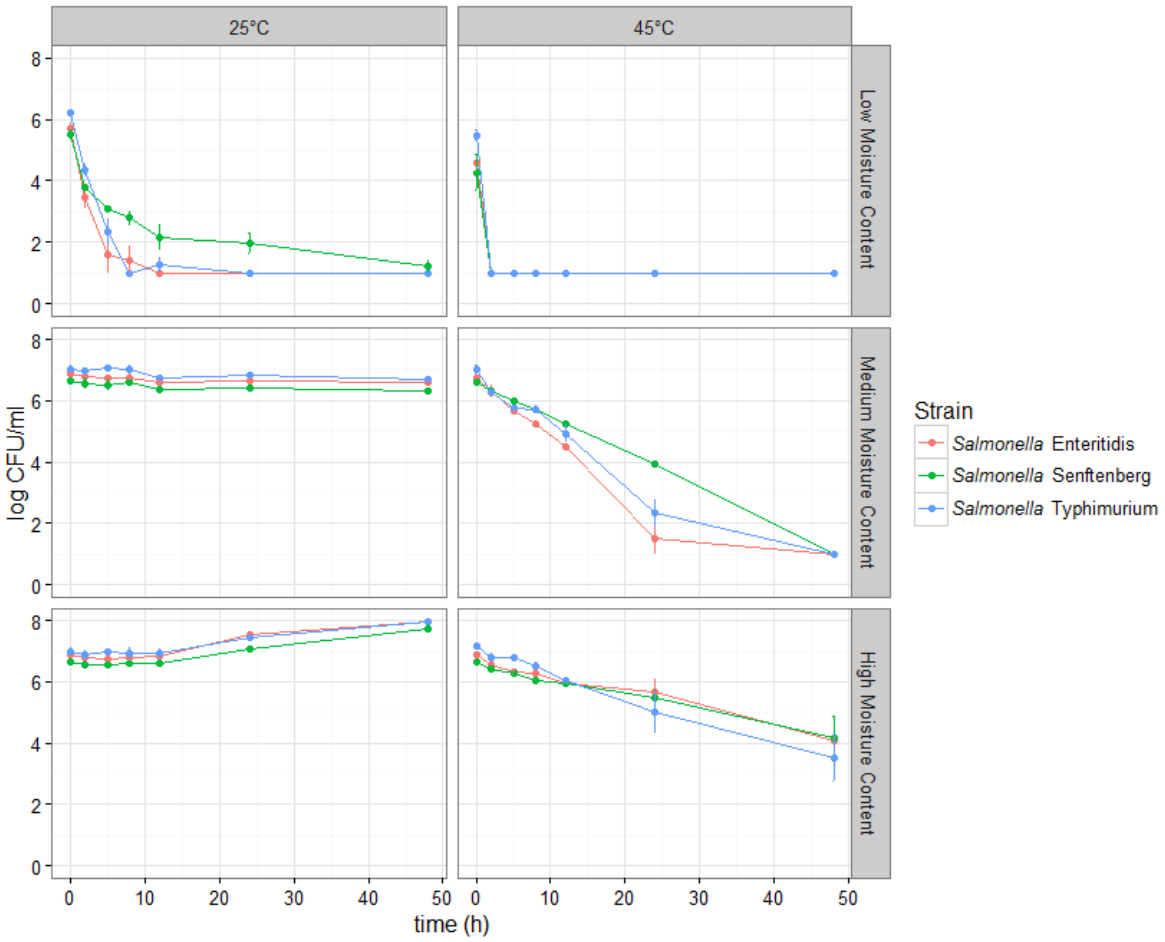

Figure 11. The survival (log CFU/ml) in XLD of *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium in 1.0% impurity level poultry fat incubated at 25˚C or 45˚C for 48 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.7 ± 0.2%; medium: 3.0 ± 0.2%; high: 4.8% ± 0.3%). Plot shows standard error bar.

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APPENDIX A

Table A.1. Effect of time and moisture content (low: 0.5%; medium: 2.2%; high: 4.5%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 0.2% impurity content, incubated at 25˚C or 45˚C for up to 24 h.

Medium	Temperature (˚C)	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	5.57^{b}	0.41	6.91^{a}	0.41	7.04^{a}	0.41	0.0215
		2	2.35^{b*}	0.41	6.61^{a}	0.41	6.65^{a}	0.41	<0.0001
		6	1.00^{b*}	0.41	6.50^{a}	0.41	6.47^{a}	0.41	<0.0001
		12	$<1.00^{b*}$	0.41	5.97^{a}	0.41	6.35^{a}	0.41	<0.0001
		24	$<1.00^{b*}$	0.41	4.84^{a*}	0.41	6.02^{a}	0.41	<0.0001
	45˚C	0	5.48^{b}	0.41	7.09^{a}	0.41	7.01^{a}	0.41	0.0103
		2	$<1.00^{b*}$	0.41	5.82^{a*}	0.41	5.98^{a}	0.41	<0.0001
		6	$<1.00^{a*}$	0.41	$<1.00^{a*}$	0.41	$<1.00^{a*}$	0.41	1.0000
		12	$<1.00^{a*}$	0.41	$<1.00^{a*}$	0.41	$<1.00^{a*}$	0.41	1.0000
		24	$<1.00^{a*}$	0.41	$<1.00^{a*}$	0.41	$<1.00^{a*}$	0.41	1.0000
XLD	25˚C	0	5.49^{b}	0.39	6.93^{a}	0.39	7.01^{a}	0.39	0.0230
		2	3.28^{b*}	0.39	6.40^{a}	0.39	6.53^{a}	0.39	<0.0001
		6	$<1.00^{b*}$	0.39	6.25^{a}	0.39	6.39^{a}	0.39	<0.0001
		12	$<1.00^{b*}$	0.39	5.67^{a*}	0.39	6.04^{a}	0.39	<0.0001
		24	$<1.00^{b*}$	0.39	4.18^{a*}	0.39	5.17^{a*}	0.39	<0.0001
	45˚C	0	5.47^{b}	0.39	6.90^{a}	0.39	6.95^{a}	0.39	0.0245
		2	$<1.00^{b*}$	0.39	4.73^{a*}	0.39	5.74^{a*}	0.39	<0.0001
		6	$<1.00^{a*}$	0.39	$<1.00^{a*}$	0.39	$<1.00^{a*}$	0.39	1.0000
		12	$<1.00^{a*}$	0.39	$<1.00^{a*}$	0.39	$<1.00^{a*}$	0.39	1.0000
		24	$<1.00^{a*}$	0.39	$<1.00^{a*}$	0.39	$<1.00^{a*}$	0.39	1.0000

SEM: standard error of the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX B

Table B.1. Effect of time and moisture content (low: 0.5%; medium: 2.1%; high: 3.9%) on *Salmonella* Enteritidis populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity content, incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	6.53[a]	0.20	6.66[a]	0.20	6.92[a]	0.20	0.3464
		2	4.95[b*]	0.20	6.88[a]	0.20	6.88[a]	0.20	<0.0001
		5	3.77[b*]	0.20	6.85[a]	0.20	6.89[a]	0.20	<0.0001
		8	3.32[b*]	0.20	6.83[a]	0.20	6.92[a]	0.20	<0.0001
		12	2.47[b*]	0.20	6.79[a]	0.20	6.96[a]	0.20	<0.0001
		24	1.20[b*]	0.20	6.77[a]	0.20	6.84[a]	0.20	<0.0001
		48	<1.00[b*]	0.20	6.56[a]	0.20	6.74[a]	0.20	<0.0001
	45˚C	0	5.82[b]	0.20	6.87[a]	0.20	6.92[a]	0.20	<0.0001
		2	1.39[b*]	0.20	6.14[a*]	0.20	6.31[a]	0.20	<0.0001
		5	1.41[b*]	0.20	4.93[a*]	0.20	5.43[a*]	0.20	<0.0001
		8	1.77[b*]	0.20	2.38[b*]	0.20	3.42[a*]	0.20	<0.0001
		12	1.20[a*]	0.20	1.40[a*]	0.20	1.45[a*]	0.20	0.6122
		24	<1.00[b*]	0.20	<1.00[b*]	0.20	1.30[a*]	0.20	0.4366
		48	<1.00[b*]	0.20	1.10[a*]	0.20	<1.00[b*]	0.20	0.9118
XLD	25˚C	0	6.37[b]	0.16	6.63[ab]	0.16	6.94[a]	0.14	0.0282
		2	4.85[b*]	0.16	6.70[a]	0.16	6.92[a]	0.14	<0.0001
		5	3.51[b*]	0.16	6.81[a]	0.16	6.82[a]	0.14	<0.0001
		8	3.19[b*]	0.16	6.72[a]	0.16	6.84[a]	0.14	<0.0001
		12	2.35[b*]	0.16	6.75[a]	0.16	6.81[a]	0.14	<0.0001
		24	<1.00[b*]	0.16	6.44[a]	0.16	6.71[a]	0.14	<0.0001
		48	<1.00[b*]	0.16	6.19[a]	0.16	6.50[a]	0.14	<0.0001
	45˚C	0	5.68[b]	0.16	6.75[a]	0.16	6.82[a]	0.16	<0.0001
		2	<1.00[b*]	0.16	5.78[a*]	0.16	6.00[a*]	0.16	<0.0001
		5	<1.00[c*]	0.16	4.19[b*]	0.16	4.78[a*]	0.16	<0.0001
		8	<1.00[c*]	0.16	1.88[b*]	0.16	3.53[a*]	0.16	<0.0001
		12	<1.00[b*]	0.16	<1.00[b*]	0.16	1.30[a*]	0.16	0.2610
		24	<1.00[a*]	0.16	<1.00[a*]	0.16	<1.00[a*]	0.16	1.0000
		48	<1.00[a*]	0.16	<1.00[a*]	0.16	<1.00[a*]	0.16	1.0000

SEM: standard error of the mean.

[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX C

Table C.1. Effect of time and moisture content (low: 0.5%; medium: 2.1%; high: 3.9%) on *Salmonella* Senftenberg populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity content, incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	5.98^{b}	0.20	6.81^{a}	0.20	6.76^{a}	0.20	0.0029
		2	4.89^{b*}	0.20	6.68^{a}	0.20	6.80^{a}	0.20	<0.0001
		5	4.16^{b*}	0.20	6.70^{a}	0.20	6.82^{a}	0.20	<0.0001
		8	3.89^{b*}	0.20	6.61^{a}	0.20	6.80^{a}	0.20	<0.0001
		12	3.58^{b*}	0.20	6.64^{a}	0.20	6.77^{a}	0.20	<0.0001
		24	3.18^{b*}	0.20	6.61^{a}	0.20	6.86^{a}	0.20	<0.0001
		48	2.71^{b*}	0.20	6.49^{a}	0.20	6.77^{a}	0.20	<0.0001
	45˚C	0	5.40^{b}	0.20	6.71^{a}	0.20	6.79^{a}	0.20	<0.0001
		2	1.48^{b*}	0.20	6.35^{a}	0.20	6.64^{a}	0.20	<0.0001
		5	$<1.00^{b*}$	0.20	5.74^{a*}	0.20	5.89^{a*}	0.20	<0.0001
		8	$<1.00^{b*}$	0.20	4.55^{a*}	0.20	5.09^{a*}	0.20	<0.0001
		12	1.68^{c*}	0.20	2.60^{b*}	0.20	3.66^{a*}	0.20	<0.0001
		24	$<1.00^{a*}$	0.20	$<1.00^{a*}$	0.20	$<1.00^{a*}$	0.20	1.0000
		48	$<1.00^{a*}$	0.20	$<1.00^{a*}$	0.20	$<1.00^{a*}$	0.20	1.0000
XLD	25˚C	0	5.73^{b}	0.16	6.64^{a}	0.16	6.59^{a}	0.16	<0.0001
		2	4.58^{b*}	0.16	6.44^{a}	0.16	6.67^{a}	0.16	<0.0001
		5	3.95^{b*}	0.16	6.42^{a}	0.16	6.66^{a}	0.16	<0.0001
		8	3.77^{b*}	0.16	6.47^{a}	0.16	6.72^{a}	0.16	<0.0001
		12	3.25^{b*}	0.16	6.41^{a}	0.16	6.60^{a}	0.16	<0.0001
		24	2.95^{b*}	0.16	6.27^{a}	0.16	6.64^{a}	0.16	<0.0001
		48	2.35^{b*}	0.16	6.08^{a*}	0.16	6.40^{a}	0.16	<0.0001
	45˚C	0	5.08^{b}	0.16	6.52^{a}	0.16	6.78^{a}	0.16	<0.0001
		2	1.38^{b*}	0.16	6.03^{a}	0.16	6.12^{a*}	0.16	<0.0001
		5	$<1.00^{b*}$	0.16	5.12^{a*}	0.16	5.43^{a*}	0.16	<0.0001
		8	1.16^{c*}	0.16	3.98^{b*}	0.16	4.62^{a*}	0.16	<0.0001
		12	$<1.00^{c*}$	0.16	2.24^{b*}	0.16	3.09^{a*}	0.16	<0.0001
		24	$<1.00^{a*}$	0.16	$<1.00^{a*}$	0.16	$<1.00^{a*}$	0.16	1.0000
		48	$<1.00^{a*}$	0.16	$<1.00^{a*}$	0.16	$<1.00^{a*}$	0.16	1.0000

SEM: standard error of the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX D

Table D.1. Effect of time and moisture content (low: 0.5%; medium: 2.1%; high: 3.9%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity content, incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	6.65[a]	0.20	6.96[a]	0.20	7.08[a]	0.20	0.2555
		2	5.45[b*]	0.20	7.08[a]	0.20	7.08[a]	0.20	<0.0001
		5	4.75[b*]	0.20	7.03[a]	0.20	7.03[a]	0.20	<0.0001
		8	4.45[b*]	0.20	6.98[a]	0.20	7.00[a]	0.20	<0.0001
		12	3.71[b*]	0.24	7.09[a]	0.20	7.01[a]	0.24	<0.0001
		24	1.62[b*]	0.20	6.83[a]	0.20	6.95[a]	0.20	<0.0001
		48	1.10[b*]	0.20	6.70[a]	0.20	6.91[a]	0.20	<0.0001
	45˚C	0	6.45[b]	0.20	7.09[a]	0.20	7.10[a]	0.20	0.0226
		2	1.28[b*]	0.20	6.18[a*]	0.20	6.56[a]	0.20	<0.0001
		5	<1.00[b*]	0.20	5.13[a*]	0.20	5.55[a*]	0.20	<0.0001
		8	<1.00[b*]	0.20	3.45[a*]	0.20	3.89[a*]	0.20	<0.0001
		12	<1.00[b*]	0.20	<1.00[b*]	0.20	1.16[a*]	0.20	0.7930
		24	<1.00[a*]	0.20	<1.00[a*]	0.20	<1.00[a*]	0.20	1.0000
		48	<1.00[a*]	0.20	<1.00[a*]	0.20	<1.00[a*]	0.20	1.0000
XLD	25˚C	0	6.74[a]	0.16	6.97[a]	0.16	6.98[a]	0.16	0.4302
		2	5.25[b*]	0.16	6.92[a]	0.16	7.06[a]	0.16	<0.0001
		5	4.07[b*]	0.16	6.94[a]	0.16	6.90[a]	0.16	<0.0001
		8	4.09[b*]	0.16	6.85[a]	0.16	6.95[a]	0.16	<0.0001
		12	2.65[b*]	0.19	6.80[a]	0.16	6.95[a]	0.19	<0.0001
		24	<1.00[b*]	0.16	6.56[a]	0.16	6.76[a]	0.16	<0.0001
		48	<1.00[b*]	0.16	6.09[a*]	0.16	6.52[a]	0.16	<0.0001
	45˚C	0	6.24[b]	0.16	7.03[a]	0.16	7.00[a]	0.16	0.0002
		2	1.28[b*]	0.16	5.78[a*]	0.16	6.15[a*]	0.16	<0.0001
		5	<1.00[b*]	0.16	4.45[a*]	0.16	4.73[a*]	0.16	<0.0001
		8	<1.00[b*]	0.16	3.19[a*]	0.16	3.47[a*]	0.16	<0.0001
		12	<1.00[b*]	0.16	<1.00[b*]	0.16	1.30[a*]	0.16	0.2610
		24	<1.00[a*]	0.16	<1.00[a*]	0.16	<1.00[a*]	0.16	1.0000
		48	<1.00[a*]	0.16	<1.00[a*]	0.16	<1.00[a*]	0.16	1.0000

SEM: standard error of the mean.

[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX E

Table E.1. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity level and low moisture content (0.5%), incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	6.53^{ab}	0.20	5.98^{a}	0.20	6.65^{a}	0.20	0.0303
		2	4.95^{a*}	0.20	4.89^{a*}	0.20	5.45^{a*}	0.20	0.0740
		5	3.77^{b*}	0.20	4.16^{ab*}	0.20	4.75^{a*}	0.20	0.0014
		8	3.32^{b*}	0.20	3.89^{ab*}	0.20	4.45^{a*}	0.20	0.0002
		12	2.47^{b*}	0.20	3.58^{a*}	0.20	3.71^{a*}	0.24	<0.0001
		24	1.20^{b*}	0.20	3.18^{a*}	0.20	1.62^{b*}	0.20	<0.0001
		48	<1.00^{c*}	0.20	2.71^{a*}	0.20	1.10^{b*}	0.20	<0.0001
	45˚C	0	5.82^{ab}	0.20	5.40^{b}	0.20	6.45^{a}	0.20	0.0006
		2	1.39^{a*}	0.20	1.48^{a*}	0.20	1.28^{a*}	0.20	0.7574
		5	1.41^{a*}	0.20	<1.00^{b*}	0.20	<1.00^{b*}	0.20	0.2156
		8	1.77^{a*}	0.20	<1.00^{b*}	0.20	<1.00^{b*}	0.20	0.0047
		12	1.20^{a*}	0.20	1.68^{a*}	0.20	<1.00^{b*}	0.20	0.0352
		24	<1.00^{a*}	0.20	<1.00^{a*}	0.20	<1.00^{a*}	0.20	1.0000
		48	<1.00^{a*}	0.20	<1.00^{a*}	0.20	<1.00^{a*}	0.20	1.0000
XLD	25˚C	0	6.37^{a}	0.16	5.73^{b}	0.16	6.74^{a}	0.16	<0.0001
		2	4.85^{ab*}	0.16	4.58^{b*}	0.16	5.25^{a*}	0.16	0.0068
		5	3.51^{b*}	0.16	3.95^{ab*}	0.16	4.07^{a*}	0.16	0.0228
		8	3.19^{b*}	0.16	3.77^{a*}	0.16	4.09^{a*}	0.16	0.0001
		12	2.35^{b*}	0.16	3.25^{a*}	0.16	2.65^{b*}	0.19	0.0001
		24	<1.00^{b*}	0.16	2.95^{a*}	0.16	<1.00^{b*}	0.16	<0.0001
		48	<1.00^{b*}	0.16	2.35^{a*}	0.16	<1.00^{b*}	0.16	<0.0001
	45˚C	0	5.68^{b}	0.16	5.08^{c}	0.16	6.24^{a}	0.16	<0.0001
		2	<1.00^{b*}	0.16	1.38^{a*}	0.16	1.28^{a*}	0.16	0.1752
		5	<1.00^{a*}	0.16	<1.00^{a*}	0.16	<1.00^{a*}	0.16	1.0000
		8	<1.00^{b*}	0.16	1.16^{a*}	0.16	<1.00^{b*}	0.16	0.6863
		12	<1.00^{a*}	0.16	<1.00^{a*}	0.16	<1.00^{a*}	0.16	1.0000
		24	<1.00^{a*}	0.16	<1.00^{a*}	0.16	<1.00^{a*}	0.16	1.0000
		48	<1.00^{a*}	0.16	<1.00^{a*}	0.16	<1.00^{a*}	0.16	1.0000

SEM: standard error of the mean.

$^{a\text{-}c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX F

Table F.1. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity level and medium moisture content (2.1%), incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	6.66^{a}	0.20	6.81^{a}	0.20	6.96^{a}	0.20	0.5367
		2	6.88^{a}	0.20	6.68^{a}	0.20	7.08^{a}	0.20	0.3256
		5	6.85^{a}	0.20	6.70^{a}	0.20	7.03^{a}	0.20	0.4842
		8	6.83^{a}	0.20	6.61^{a}	0.20	6.98^{a}	0.20	0.3838
		12	6.79^{a}	0.20	6.64^{a}	0.20	7.09^{a}	0.20	0.2353
		24	6.77^{a}	0.20	6.61^{a}	0.20	6.83^{a}	0.20	0.7036
		48	6.56^{a}	0.20	6.49^{a}	0.20	6.70^{a}	0.20	0.7153
	45˚C	0	6.87^{a}	0.20	6.71^{a}	0.20	7.09^{a}	0.20	0.3700
		2	6.14^{a*}	0.20	6.35^{a}	0.20	6.18^{a*}	0.20	0.6995
		5	4.93^{b*}	0.20	5.74^{a*}	0.20	5.13^{ab*}	0.20	0.0077
		8	2.38^{c*}	0.20	4.55^{a*}	0.20	3.45^{b*}	0.20	<0.0001
		12	1.40^{b*}	0.20	2.60^{a*}	0.20	<1.00^{c*}	0.20	<0.0001
		24	<1.00^{a*}	0.20	<1.00^{a*}	0.20	<1.00^{a*}	0.20	1.0000
		48	1.10^{a*}	0.20	<1.00^{b*}	0.20	<1.00^{b*}	0.20	0.9198
XLD	25˚C	0	6.63^{a}	0.16	6.64^{a}	0.16	6.97^{a}	0.16	0.1869
		2	6.70^{a}	0.16	6.44^{a}	0.16	6.92^{a}	0.16	0.0744
		5	6.81^{ab}	0.16	6.42^{b}	0.16	6.94^{a}	0.16	0.0395
		8	6.72^{a}	0.16	6.47^{a}	0.16	6.85^{a}	0.16	0.1864
		12	6.75^{a}	0.16	6.41^{a}	0.16	6.80^{a}	0.16	0.1342
		24	6.44^{a}	0.16	6.27^{a}	0.16	6.56^{a}	0.16	0.3934
		48	6.19^{a}	0.16	6.08^{a*}	0.16	6.09^{a*}	0.16	0.8454
	45˚C	0	6.75^{ab}	0.16	6.52^{b}	0.16	7.03^{a}	0.16	0.0577
		2	5.78^{a*}	0.16	6.03^{a}	0.16	5.78^{a*}	0.16	0.3966
		5	4.19^{b*}	0.16	5.12^{a*}	0.16	4.45^{b*}	0.16	<0.0001
		8	1.88^{c*}	0.16	3.98^{a*}	0.16	3.19^{b*}	0.16	<0.0001
		12	<1.00^{b*}	0.16	2.24^{a*}	0.16	<1.00^{b*}	0.16	<0.0001
		24	<1.00^{a*}	0.16	<1.00^{a*}	0.16	<1.00^{a*}	0.16	1.0000
		48	<1.00^{a*}	0.16	<1.00^{a*}	0.16	<1.00^{a*}	0.16	1.0000

SEM: standard error of the mean.

$^{a\text{-}c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX G

Table G.1. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity level and high moisture content (3.9%), incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	6.92[a]	0.20	6.76[a]	0.20	7.08[a]	0.20	0.4852
		2	6.88[a]	0.20	6.80[a]	0.20	7.08[a]	0.20	0.5543
		5	6.89[a]	0.20	6.82[a]	0.20	7.03[a]	0.20	0.7391
		8	6.92[a]	0.20	6.80[a]	0.20	7.00[a]	0.20	0.7673
		12	6.96[a]	0.20	6.77[a]	0.20	7.01[a]	0.24	0.6831
		24	6.84[a]	0.20	6.86[a]	0.20	6.95[a]	0.20	0.8990
		48	6.74[a]	0.20	6.77[a]	0.20	6.91[a]	0.20	0.7965
	45˚C	0	6.92[a]	0.20	6.79[a]	0.20	7.10[a]	0.20	0.5174
		2	6.31[a]	0.20	6.64[a]	0.20	6.56[a]	0.20	0.4344
		5	5.43[a*]	0.20	5.89[a*]	0.20	5.55[a*]	0.20	0.2132
		8	3.42[b*]	0.20	5.09[a*]	0.20	3.89[b*]	0.20	<0.0001
		12	1.45[b*]	0.20	3.66[a*]	0.20	1.16[b*]	0.20	<0.0001
		24	1.30[a*]	0.20	<1.00[b*]	0.20	<1.00[b*]	0.20	0.4366
		48	<1.00[a*]	0.20	<1.00[a*]	0.20	<1.00[a*]	0.20	1.0000
XLD	25˚C	0	6.94[a]	0.16	6.59[a]	0.16	6.98[a]	0.16	0.1311
		2	6.92[a]	0.16	6.67[a]	0.16	7.06[a]	0.16	0.1784
		5	6.82[a]	0.16	6.66[a]	0.16	6.90[a]	0.16	0.5064
		8	6.84[a]	0.16	6.72[a]	0.16	6.95[a]	0.16	0.5446
		12	6.81[a]	0.16	6.60[a]	0.16	6.95[a]	0.19	0.3199
		24	6.71[a]	0.16	6.64[a]	0.16	6.76[a]	0.16	0.8484
		48	6.50[a]	0.16	6.40[a]	0.16	6.52[a]	0.16	0.8239
	45˚C	0	6.82[a]	0.16	6.78[a]	0.16	7.00[a]	0.16	0.5242
		2	6.00[a*]	0.16	6.12[a*]	0.16	6.15[a*]	0.16	0.7417
		5	4.78[b*]	0.16	5.43[a*]	0.16	4.73[b*]	0.16	0.0012
		8	3.53[b*]	0.16	4.62[a*]	0.16	3.47[b*]	0.16	<0.0001
		12	1.30[b*]	0.16	3.09[a*]	0.16	1.30[b*]	0.16	<0.0001
		24	<1.00[a*]	0.16	<1.00[a*]	0.16	<1.00[a*]	0.16	1.0000
		48	<1.00[a*]	0.16	<1.00[a*]	0.16	<1.00[a*]	0.16	1.0000

SEM: standard error of the mean.

[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX H

Table H.1. Effect of time and moisture content (low: 0.7%; medium: 3.0%; high: 4.8%) on *Salmonella* Enteritidis populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity content, incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	5.84^{b}	0.17	6.86^{a}	0.17	6.88^{a}	0.17	<0.0001
		2	3.39^{b*}	0.17	6.82^{a}	0.17	6.83^{a}	0.17	<0.0001
		5	1.00^{b*}	0.17	6.77^{a}	0.17	6.78^{a}	0.17	<0.0001
		8	1.03^{b*}	0.21	6.80^{a}	0.17	6.82^{a}	0.17	<0.0001
		12	1.03^{b*}	0.21	6.83^{a}	0.17	6.88^{a}	0.17	<0.0001
		24	$<1.00^{c*}$	0.17	6.71^{b}	0.17	7.53^{a*}	0.17	<0.0001
		48	$<1.00^{c*}$	0.17	6.73^{b}	0.17	7.92^{a*}	0.17	<0.0001
	45˚C	0	4.79^{b}	0.17	6.81^{a}	0.17	6.90^{a}	0.17	<0.0001
		2	1.35^{b*}	0.17	6.35^{a}	0.17	6.59^{a}	0.17	<0.0001
		5	$<1.00^{b*}$	0.17	5.93^{a*}	0.17	6.40^{a}	0.17	<0.0001
		8	$<1.00^{b*}$	0.17	5.62^{a*}	0.17	5.96^{a*}	0.17	<0.0001
		12	$<1.00^{c*}$	0.17	4.94^{b*}	0.17	6.14^{a*}	0.17	<0.0001
		24	$<1.00^{c*}$	0.17	1.59^{b*}	0.17	5.36^{a*}	0.17	<0.0001
		48	$<1.00^{b*}$	0.17	$<1.00^{b*}$	0.17	4.91^{a*}	0.21	<0.0001
XLD	25˚C	0	5.72^{b}	0.19	6.89^{a}	0.19	6.88^{a}	0.19	<0.0001
		2	3.47^{b*}	0.19	6.79^{a}	0.19	6.79^{a}	0.19	<0.0001
		5	1.60^{b*}	0.19	6.74^{a}	0.19	6.74^{a}	0.19	<0.0001
		8	1.43^{b*}	0.19	6.74^{a}	0.19	6.80^{a}	0.19	<0.0001
		12	1.12^{b*}	0.23	6.62^{a}	0.19	6.83^{a}	0.19	<0.0001
		24	$<1.00^{c*}$	0.19	6.66^{b}	0.19	7.55^{a*}	0.19	<0.0001
		48	$<1.00^{c*}$	0.19	6.61^{b}	0.19	7.94^{a*}	0.19	<0.0001
	45˚C	0	4.59^{b}	0.19	6.75^{a}	0.19	6.86^{a}	0.19	<0.0001
		2	$<1.00^{b*}$	0.19	6.31^{a}	0.19	6.55^{a}	0.19	<0.0001
		5	$<1.00^{c*}$	0.19	5.67^{b*}	0.19	6.35^{a}	0.19	<0.0001
		8	$<1.00^{c*}$	0.19	5.23^{b*}	0.19	6.28^{a}	0.19	<0.0001
		12	$<1.00^{c*}$	0.19	4.47^{b*}	0.19	5.97^{a*}	0.19	<0.0001
		24	$<1.00^{c*}$	0.19	1.51^{b*}	0.19	5.68^{a*}	0.19	<0.0001
		48	$<1.00^{b*}$	0.19	$<1.00^{b*}$	0.19	4.06^{a*}	0.23	<0.0001

SEM: standard error of the mean.

$^{a\text{-}c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX I

Table I.1. Effect of time and moisture content (low: 0.7%; medium: 3.0%; high: 4.8%) on *Salmonella* Senftenberg populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity content, incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	5.73^{b}	0.17	6.76^{a}	0.17	6.75^{a}	0.17	<0.0001
		2	4.12^{b*}	0.17	6.65^{a}	0.17	6.64^{a}	0.17	<0.0001
		5	3.37^{b*}	0.17	6.58^{a}	0.17	6.72^{a}	0.17	<0.0001
		8	3.13^{b*}	0.17	6.64^{a}	0.17	6.80^{a}	0.17	<0.0001
		12	2.50^{b*}	0.17	6.54^{a}	0.17	6.82^{a}	0.17	<0.0001
		24	2.20^{b*}	0.17	6.66^{a}	0.17	7.18^{a}	0.17	<0.0001
		48	1.81^{c*}	0.17	6.51^{b}	0.17	7.73^{a*}	0.17	<0.0001
	45˚C	0	4.84^{b}	0.17	6.72^{a}	0.17	6.82^{a}	0.17	<0.0001
		2	1.38^{b*}	0.17	6.41^{a}	0.17	6.56^{a}	0.17	<0.0001
		5	<1.00^{b*}	0.17	6.20^{a}	0.17	6.35^{a}	0.17	<0.0001
		8	<1.00^{b*}	0.17	6.01^{a*}	0.17	6.28^{a}	0.17	<0.0001
		12	<1.00^{c*}	0.17	5.59^{b*}	0.17	6.19^{a*}	0.17	<0.0001
		24	<1.00^{c*}	0.17	4.25^{b*}	0.17	5.56^{a*}	0.17	<0.0001
		48	<1.00^{c*}	0.17	1.10^{b*}	0.17	4.44^{a*}	0.17	<0.0001
XLD	25˚C	0	5.50^{b}	0.19	6.66^{a}	0.19	6.67^{a}	0.19	<0.0001
		2	3.79^{b*}	0.19	6.57^{a}	0.19	6.56^{a}	0.19	<0.0001
		5	3.08^{b*}	0.19	6.51^{a}	0.19	6.54^{a}	0.19	<0.0001
		8	2.79^{b*}	0.19	6.60^{a}	0.19	6.60^{a}	0.19	<0.0001
		12	2.13^{b*}	0.19	6.38^{a}	0.19	6.61^{a}	0.19	<0.0001
		24	1.97^{c*}	0.19	6.41^{b}	0.19	7.08^{a}	0.19	<0.0001
		48	1.20^{c*}	0.19	6.30^{b}	0.19	7.74^{a*}	0.19	<0.0001
	45˚C	0	4.28^{b}	0.19	6.58^{a}	0.19	6.64^{a}	0.19	<0.0001
		2	<1.00^{b*}	0.19	6.31^{a}	0.19	6.42^{a}	0.19	<0.0001
		5	<1.00^{b*}	0.19	6.00^{a}	0.19	6.26^{a}	0.19	<0.0001
		8	<1.00^{b*}	0.19	5.70^{a*}	0.19	6.06^{a}	0.19	<0.0001
		12	<1.00^{c*}	0.19	5.22^{b*}	0.19	5.95^{a*}	0.19	<0.0001
		24	<1.00^{c*}	0.19	3.91^{b*}	0.19	5.49^{a*}	0.19	<0.0001
		48	<1.00^{b*}	0.19	<1.00^{b*}	0.19	4.17^{a*}	0.19	<0.0001

SEM: standard error of the mean.

$^{a\text{-}c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX J

Table J.1. Effect of time and moisture content (low: 0.7%; medium: 3.0%; high: 4.8%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity content, incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	6.14^{b}	0.17	7.10^{a}	0.17	7.00^{a}	0.17	<0.0001
		2	4.45^{b*}	0.17	7.05^{a}	0.17	6.98^{a}	0.17	<0.0001
		5	3.53^{b*}	0.17	6.95^{a}	0.17	7.03^{a}	0.17	<0.0001
		8	3.26^{b*}	0.17	6.94^{a}	0.17	7.05^{a}	0.17	<0.0001
		12	1.16^{b*}	0.17	7.02^{a}	0.17	7.12^{a}	0.17	<0.0001
		24	$<1.00^{b*}$	0.17	7.10^{a}	0.17	7.48^{a}	0.17	<0.0001
		48	$<1.00^{c*}$	0.17	6.80^{b}	0.17	7.73^{a*}	0.17	<0.0001
	45˚C	0	5.54^{b}	0.17	7.01^{a}	0.17	7.04^{a}	0.17	<0.0001
		2	1.10^{b*}	0.17	6.55^{a}	0.17	6.96^{a}	0.17	<0.0001
		5	$<1.00^{c*}$	0.17	6.27^{b*}	0.17	6.86^{a}	0.17	<0.0001
		8	$<1.00^{c*}$	0.17	5.86^{b*}	0.17	6.67^{a}	0.17	<0.0001
		12	$<1.00^{c*}$	0.17	5.31^{b*}	0.17	6.26^{a*}	0.17	<0.0001
		24	$<1.00^{c*}$	0.17	3.01^{b*}	0.17	5.00^{a*}	0.17	<0.0001
		48	$<1.00^{c*}$	0.17	1.00^{b*}	0.17	4.50^{a*}	0.21	<0.0001
XLD	25˚C	0	6.23^{b}	0.19	7.03^{a}	0.19	6.98^{a}	0.19	0.0029
		2	4.36^{b*}	0.19	6.95^{a}	0.19	6.90^{a}	0.19	<0.0001
		5	2.33^{b*}	0.19	7.09^{a}	0.19	6.98^{a}	0.19	<0.0001
		8	$<1.00^{b*}$	0.19	7.02^{a}	0.19	6.94^{a}	0.19	<0.0001
		12	1.26^{b*}	0.19	6.76^{a}	0.19	6.94^{a}	0.19	<0.0001
		24	$<1.00^{c*}$	0.19	6.81^{b}	0.19	7.45^{a}	0.19	<0.0001
		48	$<1.00^{c*}$	0.19	6.69^{b}	0.19	7.95^{a*}	0.19	<0.0001
	45˚C	0	5.48^{b}	0.19	7.02^{a}	0.19	7.17^{a}	0.19	<0.0001
		2	$<1.00^{b*}$	0.19	6.28^{a*}	0.19	6.79^{a}	0.19	<0.0001
		5	$<1.00^{c*}$	0.19	5.77^{b*}	0.19	6.79^{a}	0.19	<0.0001
		8	$<1.00^{c*}$	0.19	5.72^{b*}	0.19	6.53^{a*}	0.19	<0.0001
		12	$<1.00^{c*}$	0.19	4.92^{b*}	0.19	6.05^{a*}	0.19	<0.0001
		24	$<1.00^{c*}$	0.19	2.33^{b*}	0.19	5.00^{a*}	0.19	<0.0001
		48	$<1.00^{b*}$	0.19	$<1.00^{b*}$	0.19	3.49^{a*}	0.23	<0.0001

SEM: standard error of the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX K

Table K.1. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity level and low moisture content (0.7%), incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	5.84[a]	0.17	5.73[a]	0.17	6.14[a]	0.17	0.1987
		2	3.39[b*]	0.17	4.12[a*]	0.17	4.45[a*]	0.17	<0.0001
		5	1.00[b*]	0.17	3.37[a*]	0.17	3.53[a*]	0.17	<0.0001
		8	1.03[b*]	0.21	3.13[a*]	0.17	3.26[a*]	0.17	<0.0001
		12	1.03[b*]	0.21	2.50[a*]	0.17	1.16[b*]	0.17	<0.0001
		24	<1.00[b*]	0.17	2.20[a*]	0.17	<1.00[b*]	0.17	<0.0001
		48	<1.00[b*]	0.17	1.81[a*]	0.17	<1.00[b*]	0.17	0.0005
	45˚C	0	4.79[b]	0.17	4.84[b]	0.17	5.54[a]	0.17	0.0023
		2	1.35[a*]	0.17	1.38[a*]	0.17	1.10[a*]	0.17	0.4308
		5	<1.00[a*]	0.17	<1.00[a*]	0.17	<1.00[a*]	0.17	1.0000
		8	<1.00[a*]	0.17	<1.00[a*]	0.17	<1.00[a*]	0.17	1.0000
		12	<1.00[a*]	0.17	<1.00[a*]	0.17	<1.00[a*]	0.17	1.0000
		24	<1.00[a*]	0.17	<1.00[a*]	0.17	<1.00[a*]	0.17	1.0000
		48	<1.00[a*]	0.17	<1.00[a*]	0.17	<1.00[a*]	0.17	1.0000
XLD	25˚C	0	5.72[ab]	0.19	5.50[b]	0.19	6.23[a]	0.19	0.0181
		2	3.47[b*]	0.19	3.79[ab*]	0.19	4.36[a*]	0.19	0.0033
		5	1.60[c*]	0.19	3.08[a*]	0.19	2.33[b*]	0.19	<0.0001
		8	1.43[b*]	0.19	2.79[a*]	0.19	<1.00[c*]	0.19	<0.0001
		12	1.12[b*]	0.23	2.13[a*]	0.19	1.26[b*]	0.19	0.0004
		24	<1.00[b*]	0.19	1.97[a*]	0.19	<1.00[b*]	0.19	0.0001
		48	<1.00[b*]	0.19	1.20[a*]	0.19	<1.00[b*]	0.19	0.6746
	45˚C	0	4.59[b]	0.19	4.28[b]	0.19	5.48[a]	0.19	<0.0001
		2	<1.00[a*]	0.19	<1.00[a*]	0.19	<1.00[a*]	0.19	1.0000
		5	<1.00[a*]	0.19	<1.00[a*]	0.19	<1.00[a*]	0.19	1.0000
		8	<1.00[a*]	0.19	<1.00[a*]	0.19	<1.00[a*]	0.19	1.0000
		12	<1.00[a*]	0.19	<1.00[a*]	0.19	<1.00[a*]	0.19	1.0000
		24	<1.00[a*]	0.19	<1.00[a*]	0.19	<1.00[a*]	0.19	1.0000
		48	<1.00[a*]	0.19	<1.00[a*]	0.19	<1.00[a*]	0.19	1.0000

SEM: standard error of the mean.

[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX L

Table L.1. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity level and medium moisture content (3.0%), incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	6.86[a]	0.17	6.76[a]	0.17	7.10[a]	0.17	0.3159
		2	6.82[a]	0.17	6.65[a]	0.17	7.05[a]	0.17	0.2296
		5	6.77[a]	0.17	6.58[a]	0.17	6.95[a]	0.17	0.2925
		8	6.80[a]	0.17	6.64[a]	0.17	6.94[a]	0.17	0.4586
		12	6.83[a]	0.17	6.54[a]	0.17	7.02[a]	0.17	0.1228
		24	6.71[a]	0.17	6.66[a]	0.17	7.10[a]	0.17	0.1323
		48	6.73[a]	0.17	6.51[a]	0.17	6.80[a]	0.17	0.4314
	45˚C	0	6.81[a]	0.17	6.72[a]	0.17	7.01[a]	0.17	0.4344
		2	6.35[a]	0.17	6.41[a]	0.17	6.55[a]	0.17	0.6854
		5	5.93[a*]	0.17	6.20[a]	0.17	6.27[a*]	0.17	0.3258
		8	5.62[a*]	0.17	6.01[a*]	0.17	5.86[a*]	0.17	0.2542
		12	4.94[b*]	0.17	5.59[a*]	0.17	5.31[ab*]	0.17	0.0228
		24	1.59[c*]	0.17	4.25[a*]	0.17	3.01[b*]	0.17	<0.0001
		48	<1.00[b*]	0.17	1.10[a*]	0.17	1.00[a*]	0.17	0.8867
XLD	25˚C	0	6.89[a]	0.19	6.66[a]	0.19	7.03[a]	0.19	0.3662
		2	6.79[a]	0.19	6.57[a]	0.19	6.95[a]	0.19	0.3304
		5	6.74[a]	0.19	6.51[a]	0.19	7.09[a]	0.19	0.0848
		8	6.74[a]	0.19	6.60[a]	0.19	7.02[a]	0.19	0.2669
		12	6.62[a]	0.19	6.38[a]	0.19	6.76[a]	0.19	0.3395
		24	6.66[a]	0.19	6.41[a]	0.19	6.81[a]	0.19	0.3048
		48	6.61[a]	0.19	6.30[a]	0.19	6.69[a]	0.19	0.2907
	45˚C	0	6.75[a]	0.19	6.58[a]	0.19	7.02[a]	0.19	0.2384
		2	6.31[a]	0.19	6.31[a]	0.19	6.28[a*]	0.19	0.9881
		5	5.67[a*]	0.19	6.00[a]	0.19	5.77[a*]	0.19	0.4339
		8	5.23[a*]	0.19	5.70[a*]	0.19	5.72[a*]	0.19	0.1023
		12	4.47[b*]	0.19	5.22[a*]	0.19	4.92[ab*]	0.19	0.0168
		24	1.51[c*]	0.19	3.91[a*]	0.19	2.33[b*]	0.19	<0.0001
		48	<1.00[a*]	0.19	<1.00[a*]	0.19	<1.00[a*]	0.19	1.0000

SEM: standard error of the mean.

[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.

APPENDIX M

Table M.1. *Salmonell*a spp. populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity level and high moisture content (4.8%), incubated at 25˚C or 45˚C for up to 48 h.

Medium	Temperature (˚C)	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
			LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	25˚C	0	6.88[a]	0.17	6.75[a]	0.17	7.00[a]	0.17	0.5619
		2	6.83[a]	0.17	6.64[a]	0.17	6.98[a]	0.17	0.3672
		5	6.78[a]	0.17	6.72[a]	0.17	7.03[a]	0.17	0.3820
		8	6.82[a]	0.17	6.80[a]	0.17	7.05[a]	0.17	0.5118
		12	6.88[a]	0.17	6.82[a]	0.17	7.12[a]	0.17	0.3991
		24	7.53[a*]	0.17	7.18[a]	0.17	7.48[a]	0.17	0.2705
		48	7.92[a*]	0.17	7.73[a*]	0.17	7.73[a*]	0.17	0.6464
	45˚C	0	6.90[a]	0.17	6.82[a]	0.17	7.04[a]	0.17	0.6315
		2	6.59[a]	0.17	6.56[a]	0.17	6.96[a]	0.17	0.1788
		5	6.40[a]	0.17	6.35[a]	0.17	6.86[a]	0.17	0.0624
		8	5.96[b*]	0.17	6.28[ab]	0.17	6.67[a]	0.17	0.0110
		12	6.14[a*]	0.17	6.19[a*]	0.17	6.26[a*]	0.17	0.8723
		24	5.36[a*]	0.17	5.56[a*]	0.17	5.00[a*]	0.17	0.0615
		48	4.91[a*]	0.21	4.44[a*]	0.17	4.50[a*]	0.21	0.1781
XLD	25˚C	0	6.88[a]	0.19	6.67[a]	0.19	6.98[a]	0.19	0.4830
		2	6.79[a]	0.19	6.56[a]	0.19	6.90[a]	0.19	0.4194
		5	6.74[a]	0.19	6.54[a]	0.19	6.98[a]	0.19	0.2471
		8	6.80[a]	0.19	6.60[a]	0.19	6.94[a]	0.19	0.4224
		12	6.83[a]	0.19	6.61[a]	0.19	6.94[a]	0.19	0.4286
		24	7.55[a*]	0.19	7.08[a]	0.19	7.45[a]	0.19	0.1680
		48	7.94[a*]	0.19	7.74[a*]	0.19	7.95[a*]	0.19	0.6534
	45˚C	0	6.86[a]	0.19	6.64[a]	0.19	7.17[a]	0.19	0.1297
		2	6.55[a]	0.19	6.42[a]	0.19	6.79[a]	0.19	0.3597
		5	6.35[a]	0.19	6.26[a]	0.19	6.79[a]	0.19	0.0972
		8	6.28[a]	0.19	6.06[a]	0.19	6.53[a*]	0.19	0.2003
		12	5.97[a*]	0.19	5.95[a*]	0.19	6.05[a*]	0.19	0.9208
		24	5.68[a*]	0.19	5.49[ab*]	0.19	5.00[b*]	0.19	0.0286
		48	4.06[a*]	0.23	4.17[a*]	0.19	3.49[a*]	0.23	0.0555

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$) with Tukey adjustment.
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column per temperature with Dunnett adjustment within culture medium.