Effects of different moisture and temperature levels on *Salmonella* survival in poultry fat

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ABSTRACT: Fat products have been historically thought to have too low water activity to harbor pathogens. However, it has been recently reported that high moisture levels in fats may lead to *Salmonella* presence and growth. Limited research on strategies to eliminate pathogens in these environments is available, and the mechanisms contributing to microbial presence and growth are not yet well understood. The purpose of this research was to evaluate the effects of moisture levels and storage temperatures on the growth and survival of *Salmonella* in poultry fat. Samples were stored for 7 d at 48°C or 76°C and remaining *Salmonella* was evaluated. When poultry fat was challenged with a wet high inoculum, more than a 4 log CFU/mL difference in *Salmonella* population was observed with 1% and 3% moisture levels at 48°C after 5 d ($P < 0.05$). No differences between moisture levels ($P > 0.05$) were observed when samples were tested with a wet low inoculum. Counts below detectable limits were observed after 24 h in samples challenged at 76°C, regardless of inoculum level. When poultry fat was stored at 48°C and inoculated with low levels of *Salmonella* spp., bacterial growth was influenced only by time ($P < 0.05$) and not affected ($P > 0.05$) by moisture level. However, when poultry fat was stored at 48°C and inoculated with high levels of *Salmonella* spp., bacterial decrease was easier ($P < 0.05$) in samples containing greater moisture. This research suggests that residual moisture in containers during transportation of poultry fat largely does not affect *Salmonella* spp. growth.

Key words: dry inoculum, moisture, poultry fat, *Salmonella*, temperature, wet inoculum

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INTRODUCTION

Recycling animal coproducts into animal feed has been practiced for >45 yr as a means of converting animal waste tissue into stable, value-added materials primarily used by the animal feed and pet food industries. It is estimated that the rendering industry collects and safely processes approximately 25 million tons of animal by-products each year in the United States (Meeker and Meisinger, 2015). The ingredients produced from the rendering process range from livestock feed to fertilizer, pet food to pharmaceuticals and lard to lubricants, with the majority returned to the feed industry as high-energy fats and high-quality proteins ingredients. During the rendering process, heat is applied. The continuous cooking process used by rendering facilities has been reported to be a cycle of 40 to 90 min at 115.6 to 143.3°C (NRA, 2005). After the thermal step, fat is mechanically

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separated producing different fractions (nonfat solid and liquid fat) that are further processed. Continued improvements within the industry have implemented process control monitoring to ensure proven cook times and temperatures have been reached for inactivation of specific microorganisms deemed to be a food safety hazard (Meeker, 2006).

Although the rendering industry has an aggressive approach to animal food ingredient quality and safety by use of long cook times and high temperatures, cross-contamination with pathogenic microorganisms may still occur postprocessing. Cross-contamination after processing has been proposed as the primary factor for the presence of *Salmonella* spp. in final rendered products (Troutt et al., 2001; Denton et al., 2005; Kinley et al., 2010). A survey sponsored by the Fats and Proteins Research Foundation evaluated raw materials (animal waste tissues), crax (material from cooking/expelling process), and final rendered products for five human pathogenic bacteria, including *Salmonella* spp. (Troutt et al., 2001). The presence of *Salmonella* spp. was found in 84.5% of the raw ingredients sampled, 0% of crax samples, and 26.1% of final rendered products (Troutt et al., 2001). These results coincide with other research evaluating the presence of *Salmonella* spp. in final rendered products, including protein meals, meat and bone meals, feather meal, meat meal, and poultry meal (Denton et al., 2005; Sapkota et al., 2007; Franco, 2005; Kinley et al., 2010; Laban et al., 2014). Although the rendering process is effective at pathogen reduction as seen with 0% *Salmonella* spp. in crax (Troutt et al., 2001), it is a point-in-time mitigation technique with no residual activity. This is most problematic with animal fat, which is routinely applied to pet food after its thermal kill-step. As a result, the application of *Salmonella*-contaminated animal fat may lead to pet or human illness, even if good manufacturing practices are followed by renderers and pet food manufacturers.

Although low water activity ingredients, such as animal fat, were once thought impossible to harbor *Salmonella* spp., it is now scientifically acknowledged that small quantities of water in these ingredients may lead to contamination. It has been recently reported that high moisture levels in fats may lead to *Salmonella* and other pathogens presence and growth (Denton et al., 2005). Outbreaks involving low-moisture, high-fat foods, such as chocolate and peanut butter, reported low infectious doses of 10 to 100 *Salmonella* enterica cells, which is considerably lower than the estimated 10^4 cfu/g required to cause illness in a healthy individual (Blaser and Newman, 1982; Kapperud et al., 1993). Most rendered animal fats have definable levels of Moisture, Insolubles, and Unsaponifiables (MIU). If moisture levels rise, such as increased residual water from wet cleaning of tankers or trucks, it is plausible for *Salmonella* spp. to not only grow but thrive during transport. Microorganisms can quickly adapt to new environmental conditions and maintain viability in low-moisture foods (Gwyther et al., 2011). Therefore, the prevention of contamination is key, and control of factors influencing growth is important to understand. Limited research on strategies to eliminate pathogens in these environments is available: the mechanisms contributing to microbial presence and growth are not yet well understood. Therefore, the purpose of this research was to evaluate the effects of moisture levels and storage temperatures on the growth and survival of *Salmonella* in poultry fat overtime.

MATERIALS AND METHODS

Humane slaughter practices were followed, according to the USDA guidelines.

Samples

Poultry fat samples were obtained from a local supplier (Manhattan, KS) and tested for fat composition (average ~86.4%). Samples were stored at room temperature until the day of the experiments.

Microorganisms

Salmonella enterica Thompson (ATCC 13311), *Salmonella enterica* Newport (ATCC 6962), and *Salmonella enterica* Infantis (ATCC 51741) were selected for this study, because their association with recent pet food recalls. Cultures were grown overnight in 10-mL tryptic soy broth (BD Difco, Sparks, MD) at 37°C and sub-cultured once before cocktail preparation. A low and high inoculum (~10^6 and ~10^9 CFU/mL initial cocktail concentration) were prepared for each wet and dry inoculation procedure as explained below.

Wet Inoculation Procedure of Poultry Fat

Samples were wet inoculated using a modified method from Blessington, Theofel, and Harris (2013). Briefly, overnight *Salmonella* cultures were centrifuged for 10 min at 4000 rpm and room temperature. After centrifugation supernatants were discarded. To obtain a low concentration inoculum, pellets were resuspended in 10 mL 0.1% peptone

water (BD Difco, Sparks, MD) and from each microbial solution a 1:9 aliquot was transferred into a new tube of fresh 0.1% peptone water. Absorbance at 600 nm was measured and appropriate dilution were made to reach 0.3 value. Solutions were mixed in equal amounts to obtain a cocktail final concentration of ~10^6 CFU/mL. Pellets were instead resuspended in 5 mL 0.1% peptone water and equal amounts of each strain was mixed in order to obtain a high inoculum concentration with final population of 10^9 CFU/mL. Either low or high inoculum was then combined with samples at a liquid-to-fat ratio of 25 mL per 450 g of fat. After 1 min of agitation, the inoculated fat was divided into four different beakers of ~100 g and four different moisture levels were obtained by adding sterile water: 0%, 0.5%, 1%, and 3% moisture level. Samples were stored for 7 d at 48°C and 76°C. These two temperatures were suggested by our industrial partners, as conditions where fat can be challenged. *Salmonella* population was evaluated daily. Ten grams of the sample was preenriched in 90 mL of 1% Tween 80 Buffered Peptone (BD Difco, Sparks, MD) at 35 ± 2°C for 24 ± 2 h, enriched in both Rappaport-Vassiliadis (BD Difco, Sparks, MD) broth at 42°C for 24 h. Serial dilutions were then performed on Xylose Lysine Deoxycholaye Agar (BD Difco, Sparks, MD).

Dry Inoculation Procedure of Poultry Fat

A similar procedure as described by the Almond Board of California in Enanche et al. (2015) was followed for the preparation of the dry inoculum. Talc powder was heated at 140°C for 4 h before inoculation and tested for microbial absence. Bacteria cultures were streaked for isolation on TSA and a single colony was then transferred from lawn into 10 mL TSB at pH 7, grown aerobically from 24 h at 37°C, and transferred a second time onto TSA. After 24 h, 2 mL of peptone water was added to each plate and bacteria were harvested using a sterile spreader. Microbial solutions were combined in equal amount and 10 mL of cocktail was used to inoculate 25 g of talc. The powder was then placed into sterile Petri dish and mixed for 1 min. The concentration of cell was ~9 CFU/g. Inoculated talc was held in bags at room temperature for 24 h and allowed to dry to a final a_w of 0.44–0.46. Mortar and pestle was used to break any clumps and to obtain a fine mesh. Inoculated talc was stored up to 6 d before starting experiments. For each experiment, 2 g of inoculated talc was combined with 450 g of fat to obtain ~10^5 CFU/g in the sample (low inoculum) and 30 g of inoculated talc was instead combined with fat to obtain 10^8 CFU/mL (high inoculum). Fat was divided into four different beakers of 100 g each to obtained different moisture level, as described before. Samples were stored for 7 d at 48°C and 76°C. *Salmonella* population was evaluated daily. Ten grams of samples were preenriched in 90 mL of 1% Tween 80 Buffered Peptone (BD Difco, Sparks, MD) at 35 ± 2°C for 24 ± 2 h, enriched in both Rappaport-Vassiliadis (BD Difco, Sparks, MD) broth at 42°C for 24 h. Serial dilutions were then performed on Xylose Lysine Deoxycholaye Agar (BD Difco, Sparks, MD).

Weibull Survival Kinetics Determination

OriginPro Lab Software (version 8) was used to determine the parameters of Weibull model based on van Boekel (2002):

$$logS(t) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta}, \quad (1)$$

where the parameters α and β represent a characteristic time and curve shape, respectively. The goodness of the fit of the model was assessed using regression coefficients and least square errors. This model was applied only for samples challenged with the highest inoculum levels at 48°C, since at 76°C the count are below detectable limits after 24 h.

Statistical Analysis

Each experiment was conducted in triplicate. All the data collected were converted in log CFU/g and used to generate inactivation curves of *Salmonella*. Means and standard deviations were compared using Minitab and Excel (Microsoft Corp., Redmond, WA).

RESULTS

Figure 1 reports the data obtained when poultry fat was challenged with a high and low level of *Salmonella* wet inoculum. Wet inoculation wanted to mimic cross-contamination from moisture during transportation and storage. A 4 log CFU/mL difference in *Salmonella* population was observed with 1% and 3% moisture levels at 48°C after 5 d ($P < 0.05$; Figure 1A). The death rates were 4.8 and 17.3 d^{-1}, respectively. *Salmonella* population in samples with 0.5% moisture slowly decreased overtime and reached 2 log CFU/g at the end of the experiment (after 7 d). The control samples (no addition of water) remained constant throughout the course of the experiment. No significant difference in pathogen count was observed. When a

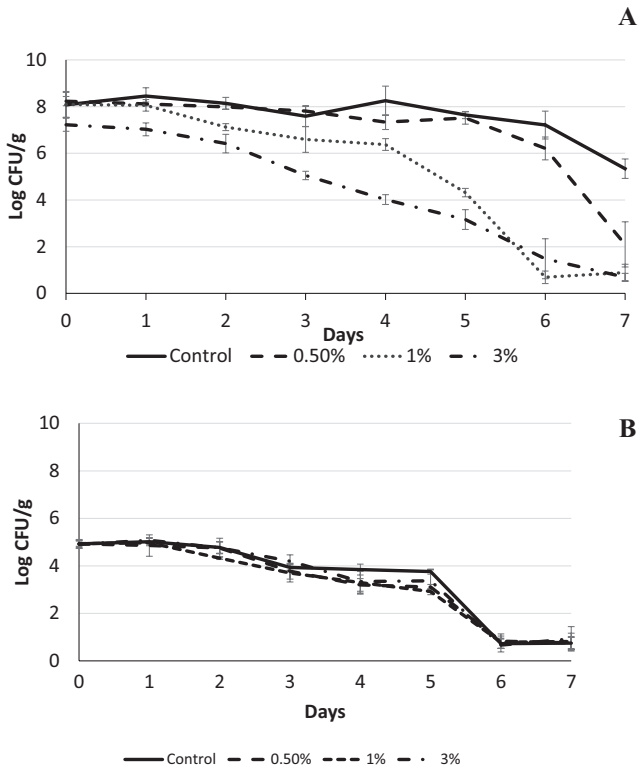

Figure 1. *Salmonella* cocktail remaining population in poultry fat samples challenged at 48°C with different wet inocula levels (high (A) and low (B), and moisture level (0%, 0.50%, 1% and 3%) over time.

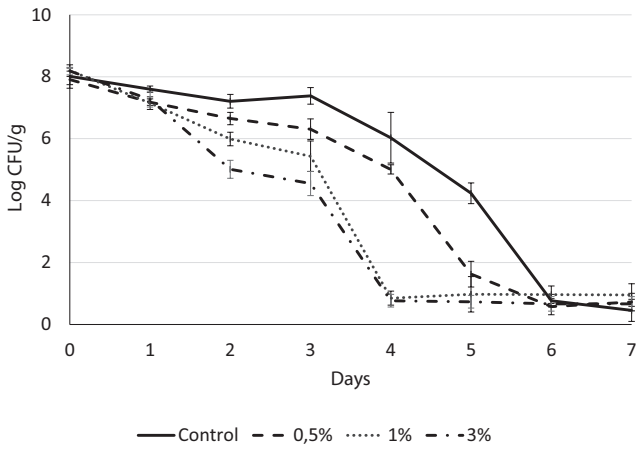

Figure 2. *Salmonella* cocktail remaining population in poultry fat samples challenged at 48°C with high dry inocula levels and different moisture levels (0%, 0.50%, 1%, and 3%) over time.

low wet inoculum was used for the challenge study, a progressive decline in *Salmonella* population was observed in all samples, including the control (Figure 1B). No statistical difference was observed between moisture levels ($P > 0.05$).

A dry inoculation method was used instead to replicate cross-contamination from insoluble fractions and samples were again challenged with different moisture levels. Figure 2 reports *Salmonella* remaining population in poultry fat samples challenged at 48°C with a high-dry inoculum level. After 4 d counts below detectable limits were recorded when samples contained 1% and 3% moisture levels when compared with the control ($P < 0.05$). The death rates were 10.5 and 8.7 d^{-1}, respectively. Similar results were observed with a high wet inoculum: moisture levels of 1% and 3% have an effect ($P < 0.05$) in reducing *Salmonella* population when samples are treated at 48°C. Nevertheless, when fat samples were challenged with a low level of *Salmonella* dry inoculum, counts were below detectable limits after 24 h. Since an enrichment procedure was followed, the author believes that the dry conditions of the inoculum could have caused too harsh of conditions for *Salmonella* to adapt to the thermal treatment of 48°C; therefore, no survivals were reported.

For the counts reported when samples were challenged at 76°C, no variations were observed between moisture levels and/or inoculum type since all counts were below detectable limits after 24 h. At these conditions only, the temperature had a significant effect ($P < 0.05$) on *Salmonell*a inactivation, and no differences among the other factors were observed.

The Weibull model was fit to explain observations and the effect of water content on survival kinetics for samples challenged with the highest inoculum levels. The model parameters β (shape parameter) and α (hazard rate or scale parameter) are given in Table 1. When the heat resistance of cells increases, the survival kinetics show a concave upward shape ($\beta < 1$), whereas a concave downward survival curve ($\beta > 1$) indicates the heat resistance of cells decreases with heating time (van Boekel, 2002). The strong correlation between the model parameters α and β is also a good indication of the reliability of the analysis and performance of the model.

DISCUSSION

Overall, a significant and rapid decrease in *Salmonella* population was observed as a function of increased temperature. Regardless of moisture level, inoculum level, or contamination level, holding poultry fat at 76°C resulted in minimal detectable *Salmonella* spp. after 24 h. When poultry fat was stored at 48°C and inoculated with low levels of *Salmonella* spp., bacterial decrease was influenced only by time and not affected by moisture level. However, when poultry fat was stored at 48°C and inoculated with high levels of *Salmonella* spp., bacterial decrease was easier in samples containing greater moisture. Our findings differ from

Table 1. Selected *Salmonella* cocktail survival kinetics in poultry fat challenged at 48 °C with different moisture levels (only high inoculum levels were included in this analysis)

			Kinetics parameters	
Inoculum condition	Moisture level	Aw	α (d)	β
Wet	0.0%	0.33 ± 0.09	5.56 ± 0.10	7.75 ± 0.15
	0.5%	0.36 ± 0.13	4.64 ± 0.92	6.44 ± 0.90
	1.0%	0.40 ± 0.07	1.62 ± 0.55	2.01 ± 0.48
	3.0%	0.51 ± 0.11	1.03 ± 0.11	1.43 ± 0.65
Dry	0%	0.33 ± 0.06	1.83 ± 0.17	2.20 ± 0.17
	0.5%	0.36 ± 0.08	1.37 ± 0.40	1.96 ± 0.38
	1.0%	0.40 ± 0.05	0.67 ± 0.42	1.45 ± 0.47
	3.0%	0.51 ± 0.04	0.37 ± 0.85	1.12 ± 0.10

those reported by (Kiel, 2018), who showed poultry fat with higher levels of moisture had greater *Salmonella* growth over time. In the Kiel study, poultry fat was stored at lower temperatures (25°C) and varying levels of impurities. We observed that moisture levels significantly affected *Salmonella* survival. When the moisture content increased, the α and β terms of the Weibull model converge to unity that the inactivation kinetics show first-order kinetics behavior. Typically, at low temperature conditions, inactivation probability increases with time, observed as large α parameter (a characteristic time), and once inactivation started damaged cells dies rapidly. Although the statistical models cannot solely be used to explain changes in inactivation mechanism, in our case, the covariate (i.e., moisture content) indicates a strong probability that at concentrations higher than 1% the survival kinetics approaches to first-order kinetics (i.e., heat resistance does not depend on time) with a decreasing hazard rate. Based on these studies together, it can be concluded that moisture affects *Salmonella* harborage and growth in poultry fat at lower temperature (at least up to 25°C) more than at moderate (48°C) or high (76°C) temperatures.

CONCLUSION

In summary, *Salmonella* harborage and growth in poultry fat can be affected by a multitude of factors, including moisture level, contamination level, temperature, and time. The present research suggests that residual moisture in containers during transportation of poultry fat largely does not affect *Salmonella* spp. growth. If contaminated with a high level of *Salmonella* spp. (10^8 cfu/mL) and held at a low temperature (48°C), moisture may influence the thermal death due to differences in water activity and water mobility kinetics.

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