Poultry Science®

Thermal inactivation kinetics of *Salmonella* and *Campylobacter* in chicken livers

Zhi Qu,[*] Devendra H. Shah ,[†,‡,2] Shyam S. Sablani ,[*] Carolyn F. Ross,[§] Sindhuja Sankaran,[*] and Juming Tang [*,1]

[*]*Department of Biological Systems Engineering, Washington State University, Pullman, WA, 99164-6120, USA;* [†]*Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, 99164-6120, USA;* [‡]*Texas Tech University School of Veterinary Medicine, Amarillo, TX, 79106, USA; and* [§]*School of Food Science, Washington State University, Pullman, WA, 99164-6120, USA*

ABSTRACT *Salmonella* and *Campylobacter* are major foodborne pathogens that cause outbreaks associated with contaminated chicken liver. Proper cooking is necessary to avoid the risk of illness to consumers. This study tested the thermal inactivation of a 4-strain *Salmonella* cocktail and a 3-strain *Campylobacter* cocktail in chicken livers separately at temperatures ranging from 55.0 to 62.5°C. Inoculated livers were sealed in aluminum cells and immersed in a water bath. The decimal reduction time (*D*-values) of *Salmonella* in chicken livers were 9.01, 2.36, 0.82, and 0.23 min at 55.0, 57.5, 60.0, and 62.5°C, respectively. The *D*-values of *Campylobacter* ranged from 2.22 min at 55.0°C to 0.19 min at 60.0°C. *Salmonella* and *Campylobacter* had similar *z*-values in chicken livers of 4.8 and 4.6°C, respectively. Chicken livers can be heated to internal temperatures of 70.0 to 73.9°C for at least 1.6 to 0.2 s to achieve a 7-log reduction of *Salmonella*. Validation tests demonstrated that heating chicken livers to internal temperatures of 70.0 to 73.9°C for 2 to 0 s resulted in a reduction of *Salmonella* exceeding 7 logs. Collectively, these data show that *Salmonella* exhibits higher heat resistance than *Campylobacter* in chicken livers. Therefore, *Salmonella* could be considered as the target pathogen when designing thermal treatments or cooking instructions for liver products. These findings will aid in designing effective thermal processing for both industrial and home cooking to eliminate *Salmonella* and *Campylobacter*, ensuring consumer safety when consuming chicken liver products.

Key words: chicken liver, *Salmonella*, *Campylobacter*, thermal inactivation, *D*-value, *z*-value

2024 Poultry Science 103:103961
https://doi.org/10.1016/j.psj.2024.103961

INTRODUCTION

Chicken liver is a byproduct of chicken, rich in nutrients such as vitamin A and iron (Ockerman and Basu, 2004). It is prepared and cooked along with other ingredients in traditional cuisine across the world, such as liver pâté, liver sausage, and fried livers. There is a growing culinary trend toward preparing chicken liver cuisines such as pâté by chefs at sit-down restaurants and by consumers at home kitchens (Porto-Fett et al., 2019; Terrasa et al., 2016). Many of the recipes on social media and/or in printed magazines instruct low-temperature short-time cooking to preserve the preferred quality attributes including texture and pink color (Food Network, 2018; New York Times, 2018). Consequently, inadequate heat transfer to the internal tissues results in the survival of foodborne pathogens, thereby increasing the risks of foodborne illness (Lanier et al., 2018). This has brought more attention to the food safety of liver and liver-related products.

The chicken liver is a potential source of foodborne pathogens, including *Salmonella* and *Campylobacter*. In a study by Jung et al. (2019), 59.4% (148 of 249) retail chicken livers were found positive for *Salmonella*, with pathogen levels ranging from 6.4 most probable number (MPN)/g to 2.4 $\log_{10}$ CFU/g. In another study, Lanier et al. (2018) reported that 67.1% (57 of 85) of chicken liver samples collected by the USDA Food Safety and Inspection Service (**USDA FSIS**) were positive for *Salmonella*. The most common serotypes of *Salmonella* recovered from chicken liver samples included *S.* Enteritidis, *S.* Heidelberg, and *S.* Typhimurium (Hanson et al., 2014; Procura et al., 2019). *Campylobacter*

Received March 24, 2024.
Accepted June 4, 2024.

[1]Co-corresponding author:
[2]Corresponding author: Devendra.Shah@ttu.edu

prevalence in chicken livers was reported to be ranging from 77 to 81% (Noormohamed and Fakhr, 2012; Strachan et al., 2012). The two main species of *Campylobacter* that are of concern to human health, *C. jejuni* and *C. coli,* have both been recovered from chicken livers (Noormohamed and Fakhr, 2012) with pathogen levels as high as 4 $\log_{10}$ CFU/g (Baumgartner and Felleisen, 2011; Nicorici and Ghoddusi, 2017).

Both *Salmonella* and *Campylobacter* are found on the surface and in the internal tissue of chicken livers (USDA FSIS, 2018). Surface contamination is likely due to unclean handling equipment and the environment in slaughterhouses (Sylte et al., 2020). In addition, chickens are naturally infected with *Salmonella* and *Campylobacter* via the fecal-oral route. These pathogens can spread from the intestine and disseminate to the internal liver tissue following intestinal colonization (Sahin et al., 2015; Sylte et al., 2020). Studies have demonstrated the presence of *Campylobacter* in the internal tissue of chicken livers after the external surface was sanitized (Whyte et al., 2006; Firlieyanti et al., 2016).

A total of 38 outbreaks related to pathogen-contaminated chicken livers were reported from 2000 to 2018 in the U.S., resulting in a total of 464 illnesses and 54 (11.6%) hospitalizations (Lanier et al., 2018). Of the 38 outbreaks, 32 (84.2%) were caused by *Campylobacter* only, 3 (7.9%) by *Salmonella* only, and 3 (7.9%) were caused by both pathogens. Similar outbreaks have also been reported in other countries (Little et al., 2010; Edwards et al., 2014; Moffatt et al., 2016). The majority of these outbreaks were related to consuming inadequately cooked chicken livers or related products at sit-down restaurants, banquet facilities, or private homes (Lanier et al., 2018).

Adequate cooking of chicken liver to eliminate *Salmonella* and *Campylobacter* is essential to achieve a microbiologically safe product. In one study, a 6.2-log reduction in *Salmonella* was achieved when inoculated chicken liver pâté was cooked to an internal temperature of 73.9°C in a water bath set at 74.9°C (Porto-Fett et al., 2019). In another study, Whyte et al. (2006) reported that pan-frying chicken livers to an internal temperature of 70°C and holding for 2 to 3 mins could inactivate all naturally occurring *Campylobacter*. However, Hutchison et al. (2015) reported that cooking chicken livers to 63°C was a critical control point for *Campylobacter*. The USDA FSIS regulates the cooking of poultry products to achieve a minimum 7-log (7D) reduction of *Salmonella*. Specifically, USDA FSIS recommends cooking poultry livers to an internal temperature of 165°F (73.9°C) without specific holding time to mitigate the food-safety risks associated with *Salmonella* and *Campylobacter* (USDA FSIS, 2005; 2020). Food Standards Australia New Zealand (**FSANZ**) and the UK Food Standards Agency (**FSA**) recommend that the whole poultry livers need to be cooked to an internal temperature of 70°C for at least 2 min to ensure they are *Campylobacter* free (FSANZ, 2018).

Currently, there are no thermal resistance parameters of *Salmonella* or *Campylobacter* in chicken livers. Consequently, it is difficult to determine alternative cooking processes at different temperatures to follow the cooking recommendations. Quantitative information on lethality is required for thermal processing development that meets the standard requirement of pathogen inactivation. Different studies have reported either *Salmonella* or *Campylobacter* as the target pathogen in chicken livers, but none of those studies has tested and compared the thermal resistance parameters of both pathogens in the same chicken liver samples (Whyte et al., 2006; Hutchison et al., 2015; Porto-Fett et al., 2019). The main objectives of this study were to determine and compare the thermal resistance of multiple strains of *Salmonella* and *Campylobacter* in chicken livers at 55.0, 57.5, 60.0, and 62.5°C and to provide evidence-based recommendations on minimum cooking conditions to achieve microbiologically safe chicken livers.

MATERIALS AND METHODS

Chicken Liver

Twenty 20-ounce packages of frozen chicken livers of the same manufacturing batch were purchased online from a reputed chicken meat production company and stored at -18°C until use. Proximate analyses of water, protein, fat, ash, and total carbohydrate content were determined three times using livers from three independent packages, following standard methods ($n = 3$) (AOAC, 2000).

Detection and Enumeration of Background Salmonella *and* Campylobacter Contamination in Chicken Liver

Two (10%) out of 20 packages of chicken livers were randomly selected and processed for the detection and quantitation of background *Salmonella* and *Campylobacter* contamination levels. The frozen livers were thawed overnight (~18 h) in the refrigerator (~4°C). Next, the livers were cut into small pieces, homogenized in the stomacher (Seward Brinkmann Stomacher 80), and approximately 25 g of the homogenized samples were mixed with 225 mL of sterile buffered peptone water (BPW) (BD/Difco, Sparks, MD) in a 13 oz Whirl-Pak bag (Nasco, Madison, WI). To increase the sensitivity of direct detection of *Salmonella,* different volumes (e.g., 0.1 mL, 1 mL) of 10-fold serial dilutions of the suspension prepared in sterile BPW were plated on duplicate Xylose Lysine Deoxycholate (**XLD**) plates (Himedia, India) and incubated at 37°C for 24 h for *Salmonella* enumeration. In addition, each liver suspension was processed for enrichment of *Salmonella* following the method described by USDA FSIS Microbiology Laboratory Guidebook 4.10 (USDA FSIS, 2019). Briefly, 1 mL of the liver suspension was transferred to 9 mL of tetrathionate broth (BD/Difco, Sparks, MD) activated with Iodine/Iodide solution (Remel, Lenexa, KS) and enriched aerobically at 37°C for 24 h and 48 h. The enrichment culture was streaked onto XLD plates and

incubated at 37°C for 24 h. Presumptive *Salmonella* colonies were tested by slide agglutination test using poly O antiserum (BD/Difco, Sparks, MD). The agglutination-positive colonies were submitted to the National Veterinary Services Laboratory, Ames, Iowa for serotyping.

For detection and enumeration of background *Campylobacter* contamination levels, 10-fold serial dilutions were plated on duplicate campy-cefex agar plates (**CCA**) (Hardy Diagnostics, Santa Maria, CA) and incubated for 48 h in a microaerophilic atmosphere (10% carbon dioxide) at 42°C. *Campylobacter* enrichment culturing was performed following the methods described by USDA FSIS Microbiology Laboratory Guidebook 41.04 (USDA FSIS, 2016). Specifically, 5 mL of the liver-BPW suspension was mixed with 5 mL of modified blood-free bolton enrichment broth (2X BF-BEB) (Hardy Diagnostics, Santa Maria, CA) and incubated for 48 to 96 h at 42°C. The enrichment cultures were streaked onto CCA and incubated in the microaerophilic atmosphere at 42°C for 48 h.

Bacterial Strains and Inoculum Preparation

The *Salmonella* strains used in this study included *S.* Heidelberg DHS-PU551 (chicken isolate), *S.* Enteritidis CDC-2010K-0968 (clinical isolate), *S.* Typhimurium SL1344 (clinical isolate), and *S.* Enteritidis strain isolated from the chicken liver in the current study. The *Campylobacter* strains used in this study included *C. jejuni* RM1221 (chicken isolate), 81-176 (clinical isolate), and F38011 (clinical isolate). The majority of the *Salmonella* and *Campylobacter* strains used in this study are known pathogens for humans and are also known to colonize chickens (Elder et al., 2016; Larson et al., 2008; Panzenhagen et al., 2018; Paul et al. 2014; Shah et al., 2017). All the *Salmonella* and *Campylobacter* test strains were maintained at -80°C in Luria-Bertani (**LB**) broth supplemented with 15% (v/v) phosphate-buffered glycerol.

The *Salmonella* cocktail was prepared by inoculating a loopful of each frozen culture onto trypticase soy agar (TSA, BD/Difco, Sparks, MD) supplemented with 0.6% yeast extract (BD/Bacto Sparks, MD) (TSAYE) for 24 h at 37°C. Next, a single isolated colony was transferred into 5 mL of trypticase soy broth (TSB, BD/Difco, Sparks, MD) supplemented with 0.6% yeast extract (TSBYE) and incubated overnight (~18 h) at 37°C with shaking at 180 rpm (Steady-Shake 757, Amerex Instruments, Concord, CA) to obtain stationary phase cultures (Mazzotta, 2001). All the strains grew equally based on the enumeration tests, thus, equal volumes of stationary-phase cultures were mixed and centrifuged at 5,000 × *g*, 4°C for 15 min (Centrifuge 5430 R, Eppendorf, Hauppauge, NY) followed by 2 washing and centrifugation with maximum recovery diluent (MRD) medium (Sigma −Aldrich, Germany) to remove the residual broth. The resulting pellet was re-suspended in MRD to obtain the 4-strain *Salmonella* cocktail to achieve final concentrations of ~10 $\log_{10}$ CFU/mL.

The *Campylobacter* cocktail was prepared by inoculating a loopful of each frozen culture onto CCA for 48 h at 42°C under microaerophilic conditions as described previously (Paul et al., 2014; USDA-FSIS, 2016). A single isolated colony from each strain was inoculated into 5 mL of 2X BF-BEB and incubated under microaerophilic conditions at 42°C for 48 h to obtain stationary phase cultures (Hazeleger et al., 2016). An aliquot of each stationary phase culture was plated onto CCA and incubated under microaerophilic conditions for an additional 48 h at 42°C. The bacterial lawns were harvested from the plate with MRD, followed by 2-step washing with MRD and centrifugation to remove the residual medium. The resulting pellet was re-suspended in MRD to obtain the 3-strain *Campylobacter* cocktail with a final concentration of approximately 10 $\log_{10}$ CFU/mL.

Liver Sample Preparation and Pathogen Inoculation

The frozen chicken livers were thawed overnight (~18h) in a refrigerator (~4°C), followed by mincing into small pieces to obtain 20 g aliquots. Next, aliquots were homogenized into a paste in a 13 oz Whirl-Pak bag at medium speed for 6 min in a stomacher (Seward Stomacher 80, UK). The pathogen inoculum was added into the liver paste at a ratio of 0.2 mL (inoculum): 20 g (liver paste) to obtain a final concentration of approximately 8 $\log_{10}$ CFU/g. The mixture was mixed manually and stomached for 4 more minutes and stored at 4°C for 20 min before isothermal treatments, to allow the bacterial cells to attach to the liver (Murphy et al., 2004).

Survival of Salmonella *and* Campylobacter *in Thermally Treated Chicken Liver*

To determine the survival of *Salmonella* and *Campylobacter* in chicken livers at different temperatures, each inoculated liver sample was subjected to the isothermal treatment using aluminum thermal death time (**TDT**) cells with an internal diameter of 31.18 mm and a height of 1.3 mm (Jin and Tang, 2019). TDT cells are optimal for testing because they have a relatively short come-up-time (**CUT**), which is the time required to reach 0.5°C less than the target temperature at the geometric center (cold spot) of the sample. In this study, the CUT was measured using a TDT cell with a 0.5 mm-diameter Type-T thermocouple that amounted through the center of the lid to the geometric center of the cell. Approximately 1.1 g of pathogen-inoculated liver samples were hermetically sealed into each TDT cell and then subjected to isothermal treatments in a water bath (Model 280, Thermo Scientific, Marietta, OH) preheated at temperatures ranging from 55.0 to 62.5°C. In this study, the CUT for liver samples at 55.0, 57.5, 60.0, and 62.5°C was determined as 30, 34, 38, and 42 s, respectively. The isothermal treatment was initiated

after CUT, i.e., the time after CUT was set to 0 min. Samples were withdrawn at each designated time interval for each isothermal treatment and transferred immediately on the ice for 2 min. Isothermal treatments at each temperature were conducted in duplicates in 3 independent experiments. Post-thermal treatment, liver samples were transferred from the TDT cells to 13 oz Whirl-Pak bags, weighed, mixed with sterile MRD, and held in the dark at room temperature for 1 h to allow the recovery of the injured cells. The 10-fold serial dilutions were plated in duplicate on XLD and CCA for the enumeration of *Salmonella* and *Campylobacter*, respectively. The XLD plates were incubated at 37°C for 24 h, and the CCA plates were incubated in the microaerophilic atmosphere at 42°C for 48 h.

Validation Test

To validate if estimated cooking conditions could achieve a 7-log reduction of *Salmonella* in chicken livers, the procedure described for the isothermal treatment and enumeration was followed. The 4-strain *Salmonella* cocktail was inoculated into chicken livers to reach an initial pathogen level of ~10 $\log_{10}$ CFU/g. The inoculated chicken livers were heated in the water bath at 70.0 for 2 s and 73.9°C for 0 seconds (treatment temperature-holding time after CUT). Three independent validation tests were conducted for each suggested cooking condition ($n = 3$).

Data Analyses

The first-order kinetic model was employed to analyze the thermal inactivation data for both *Salmonella* and *Campylobacter* at each treatment temperature (Eq. (1)) (Gaillard et al., 1998):

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D} \quad (1)$$

where t is the treatment time (min), the time after CUT was set to $t = 0$; N_0 is the initial *Salmonella* or *Campylobacter* population (CFU/g); N is the pathogen population (CFU/g) at the time t (min); D is the D-value (min), which is the time required for a 1-log or one-decimal reduction in the number of microorganisms at a certain treatment temperature (°C).

The D-value at a specific temperature was determined by plotting the $\log_{10}$ number of survivors against time. The thermal resistance constant, z-value (°C), indicates the temperature changes required to change the D-value by a factor of 10. It could be obtained through linear regression of the logarithm of D-value versus temperature as indicated in Eq. (2) (Gaillard et al., 1998):

$$z = \frac{T_2 - T_1}{log(D_1/D_2)} \quad (2)$$

The thermal inactivation data and linear regression were analyzed using Prism 8 (GraphPad Software, San Diego, CA). The goodness of fit of the linear regression was evaluated using R^2 and root mean square error (RMSE).

RESULTS

Proximate Composition of Raw Chicken Liver

The proximate composition of the raw chicken livers used in this study included water (77.9 ± 1.8%), protein (16.6 ± 0.9%), fat (2.7 ± 0.2%), ash (1.0 ± 0.1%), and carbohydrate (1.6 ± 0.2%) on a wet weight basis. The proximate composition of livers used in this study corroborated with the composition reported previously (Seong et al., 2015). The protein content of chicken liver tested in this study was within the range of reported protein content (16.8−24.1%) in different parts of the chicken meat (Pereira and Vicente, 2013; Kim et al., 2017).

Background Salmonella *and* Campylobacter *Contamination in Chicken Livers*

Only a few *Salmonella* colonies were isolated from the tested livers, indicating that the background *Salmonella* contamination in chicken livers was less than 10 CFU/g. The isolated *Salmonella* was identified as *S.* Enteritidis by the National Veterinary Services Laboratory, Ames, Iowa. *S.* Enteritidis is the predominant *Salmonella* serotype associated with human infections, and it is frequently isolated from eggs and chicken products (Altekruse et al., 2006; Shah et al., 2017; Sher et al., 2021). Consequently, *S.* Enteritidis isolated from chicken livers in the current study was also included in the 4-strain *Salmonella* cocktail for thermal inactivation tests described in the methods section. None of the liver samples processed for isolation and enumeration of *Campylobacter* yielded culture-positive results either by direct plating or by enrichments methods. Therefore, the chicken liver used in this study was regarded as *Campylobacter* negative. This is not surprising because cold storage at -20°C is known to negatively impact *Campylobacter* survival in chicken livers (Harrison et al., 2013; Gunther et al., 2019).

Thermal Inactivation Kinetics of Salmonella *and* Campylobacter

As shown in Figures 1 and 2, the $\log_{10}$ survivors of *Salmonella* and *Campylobacter* decreased linearly with heating time at respective temperatures. As expected, all D-values decreased substantially with an increase in heating temperatures. The R^2 for all the regressions was greater than 0.94. The calculated D-values of *Salmonella* were 9.01, 2.36, 0.82, and 0.23 min at 55.0, 57.5, 60.0, and 62.5°C, respectively (Table 1). *Campylobacter* had lower D-values at each of the tested temperatures when compared with *Salmonella*, with values of 2.22, 0.75, and 0.19 min at 55.0, 57.5, and 60.0°C, respectively

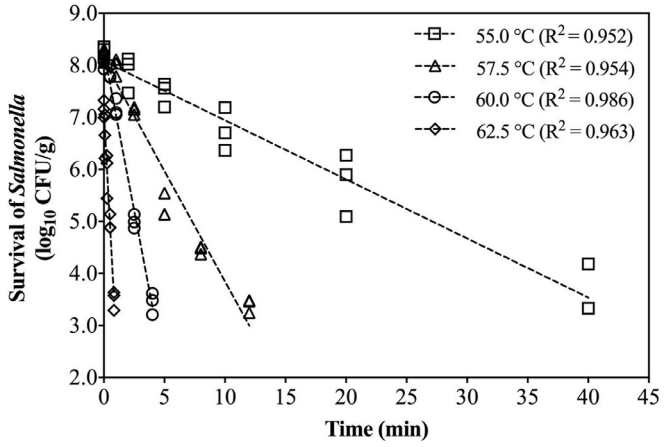

Figure 1. Thermal inactivation curves of *Salmonella* in chicken livers at 55.0, 57.5, 60.0, and 62.5°C.

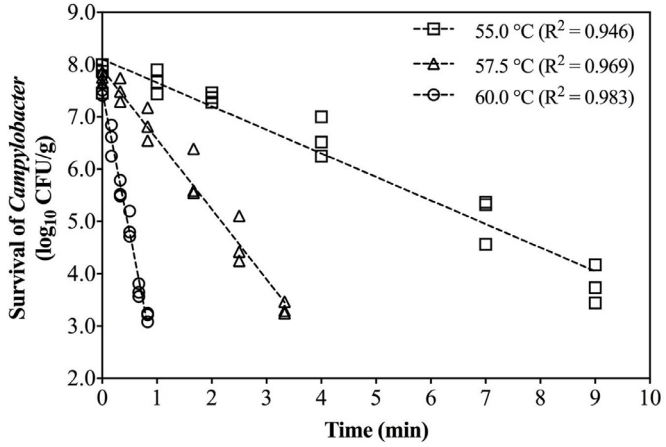

Figure 2. Thermal inactivation curves of *Campylobacter* in chicken livers at 55.0, 57.5, and 60.0°C.

(Table 1). At each heating temperature, the *D*-value of *Salmonella* was 3 to 4 times more than that of *Campylobacter*. This result indicates that *Salmonella* is more heat resistant in chicken liver than *Campylobacter*, and, as a result, requires 3 to 4 times more heating time to achieve pathogen reduction similar to *Campylobacter* at a specific temperature.

The *D*-values for both *Salmonella* and *Campylobacter* decreased linearly with increasing treatment temperatures on a semilogarithmic scale (Figure 3). The calculated *z*-value of *Salmonella* in chicken livers was 4.8°C, and similarly, *Campylobacter* had a *z*-value of 4.6°C

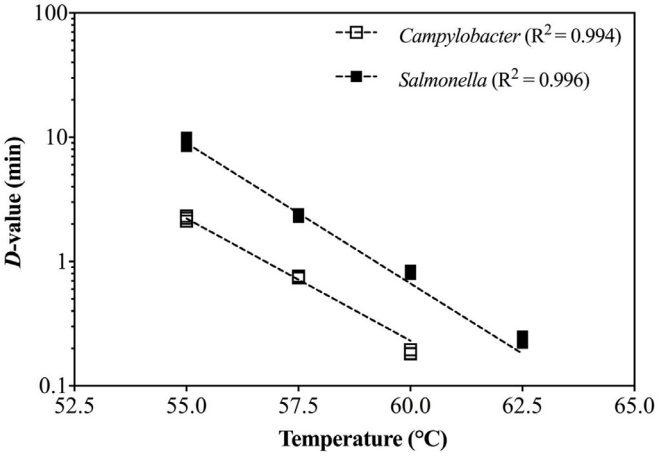

Figure 3. The semilogarithmic plot of *D*-values of *Salmonella* and *Campylobacter* in chicken livers at different treatment temperatures ($n = 3$).

(Table 1). This data demonstrates that *Salmonella* and *Campylobacter* have similar sensitivities to temperature changes in chicken livers; an increase in thermal treatment temperature of 4.8 or 4.6°C can achieve a reduction in the *D*-value of *Salmonella* or *Campylobacter* by a factor of 10, respectively.

DISCUSSION

Previous studies have revealed that the thermal resistance of *Salmonella* in different chicken meat products varies widely (Silva and Gibbs, 2012). For instance, the *D*-values of *Salmonella* in chicken nuggets and ground chicken breast ranged from 6.9 min to 0.15 min at 55.0 to 62.0°C (Mazzotta, 2000; Bucher et al., 2008). Others reported *D*-values in chicken meat ranging from 43.8 to 0.55 min at 55.0 to 65.0°C, respectively (Murphy et al., 2000; Murphy et al., 2002a,b; Murphy et al., 2004). A larger *D*-value indicates a higher heat resistance of *Salmonella* in the food matrix at the evaluated temperatures. Thus, the thermal inactivation kinetics of *Salmonella* in different chicken meat products can vary widely. This information is not currently available for chicken liver products. Consequently, we aimed to address this knowledge gap by determining the thermal inactivation kinetics (*D*- and *z*- values) of *Salmonella* in chicken livers. Our results demonstrate that the *D*-values of *Salmonella* in chicken liver (9.01−0.23 min at 55.0

Table 1. Thermal inactivation parameters for *Salmonella* and *Campylobacter* in chicken liver.

Pathogen	Temperature (°C)	*D*-value (min)[1]	95% CI upper limit of *D*-value (min)	RMSE[2]	*z*-value (°C)	R^2
Salmonella	55.0	9.01 ± 0.88	10.50	0.35	4.8	0.996
	57.5	2.36 ± 0.08	2.50	0.39		
	60.0	0.82 ± 0.03	0.87	0.21		
	62.5	0.23 ± 0.02	0.26	0.25		
Campylobacter	55.0	2.22 ± 0.11	2.40	0.35	4.6	0.994
	57.5	0.75 ± 0.01	0.77	0.28		
	60.0	0.19 ± 0.01	0.20	0.22		

[1]The *D*-values are the means of 3 independent tests ($n = 3$), expressed as mean ± SD.
[2]RMSE: the root-mean-square error, log_{10} CFU/g.

−62.5°C) are similar to the *D*-values reported in chicken nuggets and ground chicken breast previously.

The differences in the thermal resistance of *Salmonella* reported in chicken meat products could be attributed to both intrinsic and extrinsic factors. Intrinsic factor, specifically, the fat content of the food matrix significantly influences bacterial thermal resistance. The protective effects of fat on the inactivation of bacteria have been attributed to its lower heat conductivity and water activity (Murphy et al., 2003; Senhaji and Loncin, 1977). For example, in one study, the *D*-values of *Salmonella* in chicken meat with 8.45% fat content ranged from 7.08 to 1.36 min at 58.0 to 62.5°C (Juneja et al., 2001). In contrast, Murphy et al. (2002b) reported *D*-values of 22.4 to 4.6 min at 55.0 to 62.5°C in chicken tenders with 21.4% fat content. The *D*-values of *Salmonella* (9.01−0.23 min at 55.0−62.5°C) in chicken livers with a fat content of 2.68% in this study are lower than the reported *D*-values for chicken meat with a higher (8.45%) fat content (Juneja et al., 2001). Extrinsic factors, such as the sample size, geometry of the sample, and the heating containers, influence the CUT and the accuracy of thermal tolerance measurements. In this study, ~1 g of samples was sealed in an aluminum cell with a diameter of 31.18 mm and a thickness of 1.3 mm. In contrast, Murphy et al. (2002b) filled 8.5 g of chicken meat into a metal tube container with a diameter of 8.23 mm and length of 152.4 mm, Juneja (2007) heated 3 g of chicken breast in Whirl-Pak bags with a final sample thickness of 1 to 2 mm. The larger sample size, greater thickness, and low heat transfer of the heating container may result in longer CUTs during thermal treatments, thus overestimating the bacteria's thermal resistance (Bucher et al., 2008).

Porto-Fett et al. (2019) reported that treatment of *Salmonella* inoculated chicken liver pâté to 60°C reduced the *Salmonella* numbers by 1.9 log CFU/g and holding for 30 min at 60°C resulted in an additional 1.3 log CFU/g reduction, which indicates a larger *D*-value at 60°C than observed in the current study. In Porto-Fett et al.'s (2019) study, the reported fat content of the chicken liver pâté was 10.25%, and 25 g of pâté was sealed into a polypropylene centrifuge tube and heated; resulting in a CUT of 19.5 min at 60°C. The high-fat content and the relatively long CUT could be the reasons why higher thermal resistance of *Salmonella* was observed. Besides the sample matrix and heating conditions, different *Salmonella* serotypes or strains with different phenotypes can exhibit different thermal resistance. For instance, *S.* Senftenberg was widely regarded as the heat-resistant *Salmonella* serovar (Doyle and Mazzotta, 2000). We have previously reported that biofim-producing *S.* Enteritidis strains show significantly higher resistance to heat inactivation when compared with biofilm-negative strains, suggesting that biofilm-forming ability of *Salmonella* contributes to heat resistance (Villa-Rojas et al., 2017).

A limited number of studies have been conducted to determine the thermal inactivation of *Campylobacter* in chicken products. Blankenship and Craven (1982) evaluated the survival of *C. jejuni* in ground chicken breast and reported *D*-values of 2.12 to 2.25 min at 55°C and 0.79 to 0.98 min at 57°C. Others reported *D*-values of *C. jejuni* in chicken skin and chicken intestinal contents to be 1.45 to 2.20 min at 55°C and 0.18 to 0.5 min at 60°C, respectively (Oosterom et al., 1983; Yang et al., 2001). The *D*-values of *C. jejuni* strain cocktail in chicken livers from this study corroborate with the previously reported *D*-values in other chicken products.

Higher *z*-values indicate that the pathogens are more tolerant to temperature changes in a certain food matrix. The *z*-value of *Salmonella* in this study is similar to that reported in chicken nuggets (4.73−4.86°C) (Bucher et al., 2008). In contrast, the *z*-value of *Salmonella* in this study is lower than that of ground chicken breast (6.53°C) and chicken tenders (7.61°C) (Murphy et al., 2000, 2002b). Similarly, the *z*-value of *Campylobacter* in chicken livers was 25% lower when compared with the *z*-value in ground chicken meat (6.12°C) (Blankenship and Craven, 1982).

The variations in *D*- and *z*-values reported in published studies suggest that thermal inactivation parameters of different pathogens and different strains of the same pathogens need to be determined for each food formulation while calculating process lethality and in the design of processing conditions. The current results suggest that *Salmonella* is more heat resistant than *Campylobacter* in chicken livers. Therefore, the commonly practiced cooking conditions that destroy *Salmonella* in chicken livers can be expected to eliminate *Campylobacter*. Thus, *Salmonella* can be considered the target pathogen during the preparation of microbiologically-safe chicken livers. The data presented in Table 1 can be used to predict the time required at specified temperatures to achieve certain levels of *Salmonella* reductions in chicken livers. In Figure 4, the *D*-values of *Salmonella* obtained in this study were multiplied by 7 and plotted against temperature on a semilogarithmic scale to illustrate a 7-log inactivation curve. The curve shows the holding time needed under different internal temperatures to achieve a 7-log reduction of *Salmonella* in chicken livers. It is important to note that the

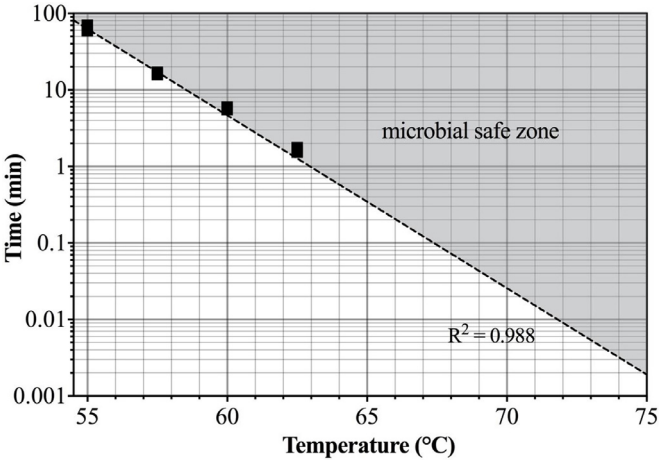

Figure 4. Semilogarithmic plot of time to achieve a 7-log reduction for *Salmonella* in chicken livers with respect to treatment temperature.

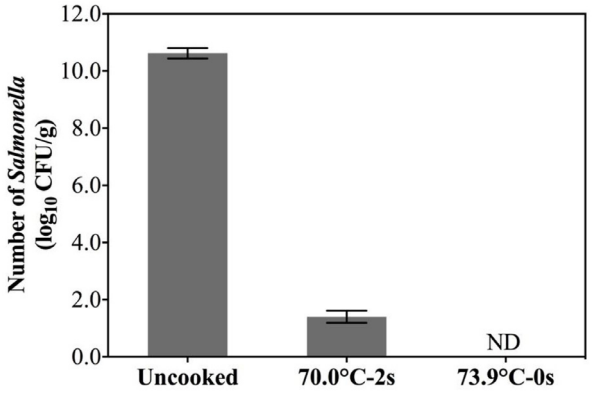

Figure 5. Inactivation of *Salmonella* in chicken livers in 2 separate thermal treatments (70.0°C-2 s and 73.9°C-0 s, treatment temperature-holding time after CUT) ($n = 3$).

temperatures should be monitored as the internal temperature of chicken liver to ensure the inactivation of the internal pathogens. The temperature and time combinations below the curve fail to achieve a 7-log reduction of *Salmonella* in chicken livers. The area above the curve is the microbiologically safe zone, where alternative treatment conditions can be considered. Based on the regression, a minimum process time at an internal temperature of 73.9°C to destroy 7 logs of *Salmonella* in chicken livers is approximately 0.2 s. This processing time is slightly higher than USDA FSIS recommendations, which do not stipulate a holding time at 73.9°C (USDA FSIS, 2020).

Based on Figure 4, a holding time of 1.6 to 0.2 s at internal temperatures of 70.0−73.9°C should be applied to achieve microbiologically safe livers with a 7-log reduction of *Salmonella* in the internal tissue of livers. To validate this, we heated the inoculated chicken livers to 70.0°C for 2 s and 73.9°C for 0 s (treatment temperature-holding time after CUT). As shown in Figure 5, the initial population of *Salmonella* in the inoculated chicken liver before thermal treatment was 10.6 $\log_{10}$ CFU/g. The population of *Salmonella* after 70.0°C-2s treatment is 1.4 $\log_{10}$ CFU/g. The number of *Salmonella* after 73.9°C-0s treatment is not detectable (**ND**, <10 CFU/g). Both suggested cooking conditions achieved more than 7 logs reduction of *Salmonella* at the cold spot of chicken liver. This result suggests that the thermal inactivation *D*- and *z*-values of *Salmonella* in chicken livers obtained in the present study can be used to develop thermal processing to effectively eliminate *Salmonella* in chicken livers at different temperatures.

CONCLUSIONS

This study was conducted to determine the thermal inactivation kinetics of *Salmonella* and *Campylobacter* in chicken livers. The results demonstrate that *Salmonella* and *Campylobacter* have similar tolerance to temperature changes, but *Salmonella* is more heat resistant than *Campylobacter* at the same temperature. Consequently, *Salmonella* can be considered as the target pathogen when evaluating and designing thermal processes for producing microbiologically safe chicken livers. The thermal resistance parameters of *Salmonella* obtained in this study can be used to guide product preparation instructions to ensure chicken liver safety against both *Salmonella* and *Campylobacter*.

ACKNOWLEDGMENTS

This work was supported by USDA AFRI (#2016-68003-24840). Zhi Qu thanks the Chinese Scholarship Council for support of her studies at Washington State University. Dr. William Lanier (USDA FSIS Senior Epidemiologist), Dr. Isabel Walls (USDA FSIS Senior Public Health Advisor and Scientific Liaison), and Michael Kuefner (Culinary Director at Thaden School) for the helpful technical discussions.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

Altekruse, S. F., N. Bauer, A. Chanlongbutra, R. DeSagun, A. Naugle, W. Schlosser, R. Umholtz, and P. White. 2006. Salmonella enteritidis in broiler chickens, United States, 2000-2005. Emerg. Infect. Dis. 12:1848–1852.

AOAC. 2000. W. Hortuntzed, Association of Official Analytical Chemists Official Methods of Analysis, 17th ed., Washington.

Baumgartner, A., and R. Felleisen. 2011. Market surveillance for contamination with thermotolerant campylobacters on various categories of chicken meat in Switzerland. J. Food Protection 74:2048–2054.

Blankenship, L. E., and S. E. Craven. 1982. Campylobacter jejuni survival in chicken meat as a function of temperature. Appli. Environ. Microbiol. 44:88–92.

Bucher, O., J. Y. D'Aoust, and R. A Holley. 2008. Thermal resistance of Salmonella serovars isolated from raw, frozen chicken nuggets/strips, nugget meat and pelleted broiler feed. Int. J. Food Microbiol. 124:195–198.

Doyle, M. E., and A. S. Mazzotta. 2000. Review of studies on the thermal resistance of Salmonellae. J. Food Protection 63:779–795.

Edwards, D. S., L. M. Milne, K. Morrow, P. Sheridan, N. Q. Verlander, R. Mulla, J. F. Richardson, A. Pender, M. Lilley, and M. Reacher. 2014. Campylobacteriosis outbreak associated with consumption of undercooked chicken liver pté in the East of England, September 2011: Identification of a dose-response risk. Epidemiol. Infect. 142:352–357.

Elder, J. R., K. L. Chiok, N. C. Paul, G. Haldorson, J. Guard, and D. H. Shah. 2016. The Salmonella pathogenicity island 13 contributes to pathogenesis in streptomycin pre-treated mice but not in day-old chickens. Gut. Pathog. 8:1–12.

Firlieyanti, A. S., P. L. Connerton, and I. F. Connerton. 2016. Campylobacters and their bacteriophages from chicken liver: The prospect for phage biocontrol. Int. J. Food Microbiol 237:121–127.

Food Network (2018). Chicken liver mousse. Accessed May 01, 2024, https://www.foodnetwork.com/recipes/alton-brown/chicken-liver-mousse-recipe-1940241.

FSANZ. (2018). Campylobacter in chicken liver, Accessed May 01, 2024, Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/scienceandtechnical/campylobacter_in_chicken_liver.pdf.

Gaillard, S., I. Leguerinel, and P. Mafart. 1998. Model for combined effects of temperature, pH and water activity on thermal inactivation of Bacillus cereus spores. J. Food Sci. 63:887–889.

Gunther, N. W., A. Abdul-Wakeel, O. J. Scullen, and C. Sommers. 2019. The evaluation of gamma irradiation and cold

storage for the reduction of Campylobacter jejuni in chicken livers. Food Microbiol. 82:249–253.
Hanson, H., W. T. Hancock, C. Harrison, L. Kornstein, H. Waechter, V. Reddy, J. Luker, M. Malavet, P. Huth, L. Gieraltowski, and S. Balter. 2014. Creating student sleuths: How a team of graduate students helped solve an outbreak of Salmonella Heidelberg infections associated with Kosher broiled chicken livers. J. Food Protection 77:1390–1393.
Harrison, D., J. E. L. Corry, M. A. Tchórzewska, V. K. Morris, and M. L. Hutchison. 2013. Freezing as an intervention to reduce the numbers of campylobacters isolated from chicken livers. Lett. Appl. Microbiol. 57:206–213.
Hazeleger, W. C., W. F. Jacobs-Reitsma, and H. M. W.de Besten. 2016. Quantification of growth of *Campylobacter* and extended spectrum β-lactamase producing bacteria sheds light on black box of enrichment procedures. Front. Microbiol. 7:1–9.
Hutchison, M., D. Harrison, I. Richardson, and M. Tchórzewska. 2015. A method for the preparation of chicken liver pâté that reliably destroys campylobacters. Int. J. Environ. Res. Public Health 12:4652–4669.
Jin, Y., and J. Tang. 2019. Improved design of aluminum test cell to study the thermal resistance of Salmonella enterica and Enterococcus faecium in low-water activity foods. Food Cntrl 104:343–348.
Juneja, V. K., B. S. Eblen, and G. Ramsom. 2001. Thermal inactivation of Salmonella spp. in chicken broth, beef, pork, turkey, and chicken: Determination of D- and Z-values. J. Food Sci. 66:146–152.
Juneja, Vijay K. 2007. Thermal inactivation of Salmonella spp. in ground chicken breast or thigh meat. Int. J. Food Sci. Technol. 42:1443–1448.
Jung, Y., A. C. S. Porto-Fett, B. A. Shoyer, E. Henry, L. E. Shane, M. Osoria, and J. B. Luchansky. 2019. Prevalence, levels, and viability of Salmonella in and on raw chicken livers. J. Food Prot. 82:834–843.
Kim, H., H. W. Do, and H. Chung. 2017. A comparison of the essential amino acid content and the retention rate by chicken part according to different cooking methods. Korean J. Food Sci. Anim. Resour. 37:626–634.
Lanier, W. A., K. R. Hale, A. L. Geissler, and D. Dewey-Mattia. 2018. Chicken liver-associated outbreaks of campylobacteriosis and salmonellosis, United States, 2000-2016: Identifying opportunities for prevention. Foodborne Pathog. Dis. 15:726–733.
Larson, C. L., D. H. Shah, A. S. Dhillon, D. R. Call, S. Ahn, G. J. Haldorson, C. Davitt, and M. E. Konkel. 2008. Campylobacter jejuni invade chicken LMH cells inefficiently and stimulate differential expression of the chicken CXCLi1 and CXCLi2 cytokines. Microbiology (N. Y) 154:3835–3847.
Little, C. L., F. J. Gormley, N. Rawal, and J. F. Richardson. 2010. A recipe for disaster: Outbreaks of campylobacteriosis associated with poultry liver pâté in England and Wales. Epidemiol. Infect. 138:1691–1694.
Mazzotta, A. S. 2001. Thermal inactivation of stationary-phase and acid-adapted Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes in fruit juices. J. Food Prot. 64:315–320.
Mazzotta, A. S. 2000. D- and z-values of Salmonella in ground chicken breast meat. J. Food Saf. 20:217–223.
Moffatt, C. R. M., A. Greig, M. Valcanis, W. Gao, T. Seemann, B. P. Howden, and M. D. Kirk. 2016. A large outbreak of Campylobacter jejuni infection in a university college caused by chicken liver pâté, Australia, 2013. Epidemiol. Infect. 144:2971–2978.
Murphy, R. Y., L. K. Duncan, B. L. Beard, and K. H. Driscoll. 2003. D and z values of Salmonella, Listeria innocua, and Listeria monocytogenes in fully cooked poultry products. J. Food Sci. 68:1443–1447.
Murphy, R. Y., L. K. Duncan, M. E. Berrang, J. A. Marcy, and R. E. Wolfe. 2002. Thermal inactivation D- and Z-values of Salmonella and Listeria innocua in fully cooked and vacuum packaged chicken breast meat during postcook heat treatment. Poult. Sci. 81:1578–1583.
Murphy, R. Y., L. K. Duncan, E. R. Johnson, M. D. Davis, and J. N. Smith. 2002. Thermal inactivation D- and z-values of Salmonella serotypes and Listeria innocua in chicken patties, chicken tenders, franks, beef patties, and blended beef and turkey patties. J. Food Prot. 65:53–60.
Murphy, R. Y., B. P. Marks, E. R. Johnson, and M. G. Johnson. 2000. Thermal inactivation kinetics of Salmonella and Listeria in g round chicken breast meat and liquid medium. J. Food Sci. 65:706–710.
Murphy, R. Y., T. Osaili, L. K. Duncan, and J. A. Marcy. 2004. Thermal inactivation of Salmonella and Listeria monocytogenes in ground chicken thigh/leg meat and skin. Poult. Sci. 83:1218–1225.
New York Times (2018). Chicken liver pate. Accessed May 01, 2024, https://cooking.nytimes.com/recipes/1015975-chicken-liver-pate.
Nicorici, G., and H. B. Ghoddusi. 2017. Prevalence of Campylobacter contamination in raw chicken and chicken liver at retail. Food Safety Magazine 23:44–73.
Noormohamed, A., and M. K. Fakhr. 2012. Incidence and antimicrobial resistance profiling of campylobacter in retail chicken livers and gizzards. Foodborne Pathog. Dis. 9:617–624.
Ockerman, H. W., and L. Basu. 2004. By-products. Pages 104−112 in Animal By-Product Processing & Utilization.
Oosterom, J., G. J. A. De Wilde, E. De Boer, L. H. De Blaauw, and H. Karman. 1983. Survival of Campylobacter jejuni during Poultry Processing and Pig Slaughtering. J. Food Prot. 46:702–706.
Panzenhagen, P. H. N., N. C. Paul, C. A. Conte, R. G. Costa, D. P. Rodrigues, and D. H. Shah. 2018. Genetically distinct lineages of Salmonella Typhimurium ST313 and ST19 are present in Brazil. International Journal of Medical Microbiology 308:306–316.
Paul, N. C., S. Al-Adwani, R. Crespo, and D. H. Shah. 2014. Evaluation of passive immunotherapeutic efficacy of hyperimmunized egg yolk powder against intestinal colonization of Campylobacter jejuni in chickens. Poult. Sci. 93:2779–2787.
Pereira, P. M.de C. C., and A. F.dos R. B. Vicente. 2013. Meat nutritional composition and nutritive role in the human diet. Meat. Sci. 93:586–592.
Porto-Fett, A. C. S., B. A. Shoyer, L. E. Shane, M. Osoria, E. Henry, Y. Jung, and J. B. Luchansky. 2019. Thermal inactivation of salmonella in pâté made from chicken liver. J. Food Prot. 82:980–987.
Procura, F., D. J. Bueno, S. B. Bruno, and A. D. Rogé. 2019. Prevalence, antimicrobial resistance profile and comparison of methods for the isolation of salmonella in chicken liver from Argentina. Food Research International 119:541–546.
Sahin, O., I. I. Kassem, Z. Shen, J. Lin, G. Rajashekara, and Q. Zhang. 2015. Campylobacter in Poultry: Ecology and Potential Interventions. Avian Dis. 59:185–200.
Senhaji, A. F., and M. Loncin. 1977. The protective effect of fat on the heat resistance of bacteria (I). Int. J. Food Sci. Technol. 12:203–216.
Seong, P. N., S. H. Cho, K. M. Park, G. H. Kang, B. Y. Park, S. S. Moon, and H. Van Ba. 2015. Characterization of chicken by-products by mean of proximate and nutritional compositions. Korean J. Food Sci. Anim. Resour. 35:179–188.
Shah, D. H., N. C. Paul, W. C. Sischo, R. Crespo, and J. Guard. 2017. Population dynamics and antimicrobial resistance of the most prevalent poultry-associated Salmonella serotypes. Poult. Sci. 96:687–702.
Sher, A. A., B. E. Mustafa, S. C. Grady, J. C. Gardiner, and A. M. Saeed. 2021. Outbreaks of foodborne Salmonella enteritidis in the United States between 1990 and 2015: An analysis of epidemiological and spatial-temporal trends. International Journal of Infectious Diseases 105:54–61.
Silva, F. V. M., and P. A. Gibbs. 2012. Thermal pasteurization requirements for the inactivation of Salmonella in foods. Food Research International 45:695–699.
Strachan, N. J. C., M. MacRae, A. Thomson, O. Rotariu, I. D. Ogden, and K. J. Forbes. 2012. Source attribution, prevalence and enumeration of Campylobacter spp. from retail liver. Int. J. Food Microbiol. 153:234–236.
Sylte, M. J., D. C. Shippy, B. L. Bearson, and S. M. D. Bearson. 2020. Detection of Campylobacter jejuni liver dissemination in experimentally colonized turkey poults. Poult. Sci. 99:4028–4033.
Terrasa, A. M., M. Dello Staffolo, & M. C. Tomás. 2016. Nutritional improvement and physicochemical evaluation of liver pâté formulations. *LWT - Food Science and Technology*, 66:678−684.
USDA FSIS. (2005). Risk assessment of the impact of lethality standards on salmonellosis from ready-to-eat meat and poultry products

final report. Accessed May 01, 2024, Retrieved from http://www.fsis.usda.gov/wps/portal/fsis/topics/science/risk-assessments.

USDA FSIS. (2016). Isolation and identification of Campylobacter jejuni /coli / lari from poultry rinse, sponge and raw product samples. *Microbiology Laboratory Guidebook*, 41.04.

USDA FSIS. (2018). Minimizing the risk of Campylobacter and Salmonella illnesses associated with chicken liver, (July), 1−9.

USDA FSIS. (2019). Isolation and identification of Salmonella from meat, poultry, pasteurized egg, and siluriformes (fish) products and carcass and environmental sponges. *Microbiology Laboratory Guidebook*, 4.10.

USDA FSIS. (2020). Chicken liver - resources for illness prevention. Accessed Jan. 5, 2021, from https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/chicken-liver.

Villa-Rojas, R., M. J. Zhu, N. C. Paul, P. Gray, J. Xu, D. H. Shah, and J. Tang. 2017. Biofilm forming Salmonella strains exhibit enhanced thermal resistance in wheat flour. Food Control 73:689–695.

Whyte, R., J. A. Hudson, and C. Graham. 2006. Campylobacter in chicken livers and their destruction by pan frying. Lett. Appl. Microbiol. 43:591–595.

Yang, H., Y. Li, and M. G. Johnson. 2001. Survival and death of Salmonella Typhimurium and Campylobacter jejuni in processing water and on chicken skin during poultry scalding and chilling. J. Food Prot. 64:770–776.