SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 22, NUM. 04 2026
doi: 10.14808/sci.plena.2026.046201

Predictive model of growth and survival of *Salmonella* Typhimurium in pork ham: Pathogen adaptation to different temperatures

Modelo preditivo de crescimento e sobrevivência de *Salmonella* Typhimurium em presunto suíno: Adaptação do patógeno a diferentes temperaturas

G. S. Sodre[1] *; P. F. M. de Sousa[1]; R. M. Senado[2]; A. P. P. O. Sampaio[1]; J. B. da Silva[1]; J. S. Lima[1]

[1]*Laboratory of Food Hygiene and Quality, Institute of Veterinary Medicine, Federal University of Pará, 68740-970, Castanhal-Pará, Brazil*
[2]*University of the Amazon, 68743-010, Castanhal-Pará, Brazil*

**gabrielsodre44@gmail.com*
(Recebido em 16 de março de 2025; aceito em 28 de março de 2026)

Processed foods are quite attractive due to their practicality and flavor. The processing methods promise product quality and safety, with ham standing out, as it is highly consumed by the population. However, despite several intrinsic and extrinsic barriers, this food remains vulnerable to microbial contamination due to countless factors. In this study, we aimed to investigate the kinetics and survival of *Salmonella* Typhimurium (*S.* Typhimurium) in baked pork ham and sliced ham, as well as its death kinetics. We simulated contamination in baked pork and sliced ham and the bacterial concentrations were analyzed at 0 (inoculation time), 6, 12, 24, 48, and 72 h, at 8 ºC and 30 ºC. Furthermore, an inactivation treatment was carried out with constant temperatures at 60 ºC and 65 ºC for 0 (moment of inoculation), 2.5, 5, 7.5,10, and 15 min. The test results demonstrated that the strain survived at both temperatures (8 ºC and 30 ºC). However, the highest bacterial concentrations were found in food treated at 30º C. In addition, the strain was able to survive at 60 °C for the 15 min (D value: 4.3 min) of treatment. However, the pathogen was inactivated in 5 min at 65 ºC (D value: 0.85 min). The present study demonstrated the survival of *S.* Typhimurium at 8º C, showing that as the temperature increases (30 º C) its metabolism accelerates, which favors its growth in ham. This suggests that this pathogen adapts to different conditions in ham.
Keywords: predictive model, extrinsic factors, microbial behavior.

Alimentos processados são bastante atrativos devido à sua praticidade e sabor. Os métodos de processamento prometem qualidade e segurança do produto, com destaque para o presunto, por ser altamente consumido pela população. No entanto, apesar de diversas barreiras intrínsecas e extrínsecas, esse alimento continua vulnerável à contaminação microbiana devido a inúmeros fatores. Neste estudo, objetivamos investigar a cinética e sobrevivência de *Salmonella* Typhimurium (*S.* Typhimurium) em presunto suíno assado e presunto fatiado, bem como sua cinética de morte. Simulamos a contaminação em presunto suíno fatiado e as concentrações bacterianas foram analisadas em 0 (tempo de inoculação), 6, 12, 24, 48 e 72 h, a 8ºC e 30ºC. Além disso, foi realizado um tratamento de inativação com temperaturas constantes de 60 ºC e 65 ºC por 0 (momento de inoculação), 2.5, 5, 7.5, 10 e 15 min. Os resultados dos testes demonstraram que a cepa sobreviveu em ambas as temperaturas (8 ºC e 30 ºC). No entanto, as maiores concentrações bacterianas foram encontradas em alimentos tratados a 30 ºC. Além disso, a cepa foi capaz de sobreviver a 60 °C durante os 15 min (valor D: 4,3 min) de tratamento. No entanto, o patógeno foi inativado em 5 min a 65 ºC (valor D: 0,85 min). O presente estudo demonstrou a sobrevivência de *S.* Typhimurium a 8º C, mostrando que à medida que a temperatura aumenta (30 ºC) seu metabolismo acelera, o que favorece seu crescimento no presunto. Isso sugere que esse patógeno se adapta a diferentes condições no presunto.
Palavras-chave: modelo preditivo, fatores extrínsecos, comportamento microbiano.

1. INTRODUCTION

Ham pork is very appreciated by the general population and, like other processed foods, has been increasingly consumed, due its practicality and characteristic flavor. However, these foods

are not free from microbial contamination, even with technological advances to ensure product quality and food safety [1-4].

Ham contamination can occur during processing and post-processing, usually occurring due to lack of hygiene among handlers, biosafety failures, inadequate maintenance of infrastructure, and poorly sanitized equipment [5, 6]. Care during transportation, storage, and fractionation are also very important for bacterial contamination, especially contamination with *Salmonella* spp. [7].

Bacteria of the genus *Salmonella* are gram-negative rods and facultative anaerobes, belonging to the Enterobacteriaceae family. Among the representatives of this genus, the species *Salmonella enterica* subspecies *enterica* is the most prominent, containing more than 2,500 serovars. Of these, *Salmonella* Typhimurium (*S*. Typhimurium) is the most important, as one of the most prevalent serovars associated with foodborne disease outbreaks (FBDOs) [8, 9].

Due to the possibility of contamination of pork ham with *Salmonella* spp., it is crucial to understand its growth and survival in this food, to reduce the numbers of FBDO cases. Moreover, there are countless factors that favor or inhibit microbial growth, such as nutritional composition, accompanying microbiota, pH, water activity, and storage temperatures [4, 10, 11].

Resolution No. 216, dated September 15, 2004, from the National Surveillance Agency Health (ANVISA) [12], provides guidelines on good practices in food preparation services, establishing a series of criteria in relation to temperature. One example is the thermal treatment of frozen food, which must be thawed under refrigeration (below 5 ºC) and subsequently subjected to temperatures of at least 70 ºC to guarantee the safety of the product.

Furthermore, during storage and transport, the temperature must be constantly monitored. Based on in this, it is clear how critical temperature control is in these cases, as required by law, to control microbial growth in food [12]. In this context, studies that evaluate the behavior of pathogenic microorganisms and their growth kinetics in food matrices are needed. In this way, biological control measures can be adopted and outbreaks or even public health crises can be avoided [13].

The objective of this study was to describe the growth kinetics of *Salmonella enterica* serovar Typhimurium in backed pork ham and sliced ham, under different storage temperatures (8 ºC and 30 º C), including its growth parameters (generation time and growth rate) and thermal inactivation of the microorganism under constant temperatures (60 ºC and 65 ºC).

2. MATERIALS AND METHODS

2.1 Sample preparation

The procedures adopted in this study were based Lima et al. (2021) [10], Sabike et al. (2015) [14], and Zaher and Fujikawa (2011) [15].

The baked and sliced ham samples were obtained from the local market, preferably in vacuum packaging, with a federal inspection seal, and transported in isothermal boxes to the Food Hygiene and Quality Laboratory, Federal University of Pará (UFPA), Castanhal campus, PA, Brazil. The ham samples were fractioned into 25 g portions for experimental contamination with *S.* Typhimurium. It is worth noting that this amount of food was chosen based on the principles outlined in Normative Instruction - IN N$^{o.}$ 161, of July 1, 2022 of the ANVISA [16], which recommends the absence of *Salmonella* spp. in every 25 g of food.

Under aseptic conditions, the samples were deposited in Styrofoam trays, similar to those used in supermarkets, covered with plastic film, previously sterilized under UV radiation. In addition, a 25 g portion of ham was designated for microbiological analysis according to ISO 6579-1:2002 for safety control, ensuring that the study samples were not contaminated with field strains.

2.2 Preparation of *S.* Typhimurium strain

S. Typhimurium (ATCC 14028) was cultivated in 5 mL of brain-heart infusion (BHI) broth and incubated at 37 ºC for 18-24 h. Then, it was plated for exhaustion in Xylose Lysine Deoxycholate (XLD) agar and incubated at 37ºC for 18-24 h to obtain isolated colonies for subsequent contamination of the ham samples. In all stages of the procedure, strain purity was controlled through bacterioscopy with Gram staining.

2.3 Standardization of bacterial inoculum

Standardization of *S.* Typhimurium inoculum was carried out with different concentrations to determine the ideal initial bacterial concentration for modeling pathogen growth in the food in question. For this, the strain previously cultivated in BHI broth was serially diluted in saline solution (0.85%) and different dilutions were inoculated into the ham using aliquots of 1 mL for every 25 g of food. The initial *S.* Typhimurium inoculum concentrations tested ranged from 10^1 to 10^9 CFU/ mL. Subsequently, the samples were incubated at 37 °C for 24 h, after that period, titration was performed in XLD agar, and bacterial counting was done to determine the optimal initial inoculum concentration for subsequent tests.

2.4 Experimental contamination and growth kinetics of *S.* Typhimurium

A 1 mL aliquot of the inoculum previously standardized (10^6 CFU/mL) was used for contamination of the ham samples (25 g per sample), which remained in contact with the suspension for 30 min at room temperature to allow bacterial cell adhesion to the food. Then, the samples were incubated at 8 ºC and 30 ºC. The food was analyzed at: 0 (moment of inoculation), 6, 12, 24, 48, and 72 h.

Determination of *S.* Typhimurium at each time point was determined by adding the ham samples to 225 mL of peptone saline solution (PSS). Serial dilutions (10^{-1} to 10^{-4}) were then performed in saline solution (0.85%), and 100 µL aliquots of each dilution were inoculated in XLD and Salmonella-Shigella (SS) agar plates using a Drigalsky loop. The plates were incubated at 37 ºC for 24 h. After growth, the colonies were counted and the values were expressed as CFU/g.

2.5 Generation time and growth rate

The calculations of the average generation time (G) and specific growth rate (µ) were carried out using the values obtained during the first 24 h of modeling, between the exponential phase and the beginning of the stationary growth phase. The equations for determining these variables, described by Lemos et al. (1996) [17], are shown below:

(1) Average Generation Time (G)

$$G = \frac{t}{3{,}322(log\ log\ Nf\ - logNi)}$$

(2) Specific Growth Rate (µ)

$$\mu = \frac{ln\ ln\ Nf - ln\ ln\ Ni}{t}$$

t = time (min)
Ni = Initial number of cells ($CFU.mL^{-1}$)
Nf = Final number of cells ($CFU.mL^{-1}$)

2.6 Thermal killing / inactivation kinetics and D value

A 1 mL aliquot of the *S.* Typhimurium inoculum, previously standardized (10^8 CFU/mL), was used to contaminate the 25 g samples of ham, which remained in contact with the suspension for 30 min at room temperature to allow bacterial cell adhesion to the food.

The samples were packaged in sterile plastic bags and immersed in bain-marie at 60 ºC and 65 ºC in time intervals of 2.5, 5, 7, 5, 10, and 15 min. After each time interval, the samples were added to an ice bath (8 ºC) to stop the treatment. Subsequently, the food fractions were added to 225 mL of SSP, homogenized, and serial dilutions (10^{-1} to 10^{-3}) were performed in a saline solution (0.85%). Aliquots of 100µL of each dilution were inoculated XLD and SS agar plates using a Drigalsky loop, and incubated at 37 °C for 18-24 h.

After recording the microbial concentrations at each inactivation time, the D value was calculated based on the slope of the linear regression line, fitted to the data and modeled using the equation generated by the test. The D value represents the time (in minutes) required for a 90% reduction in a bacterial population at a constant temperature or, that is, the time required for a 1 log reduction in CFU of the bacteria.

2.7 Statistical analysis

The results obtained were submitted to descriptive and analytical statistical analysis using Microsoft Office Excel and Bioestat v. 5.0 software. The averages of the treatments at different temperatures of the growth kinetics were compared using a paired t-test, with a significance level of 5%. In addition, linear regression analysis was applied to the bacterial death kinetics essays.

3. RESULTS AND DISCUSSION

The results demonstrated that *Salmonella* Typhimurium was able to survive and grow at 8 and 30 ºC in sliced ham, following contamination with an initial inoculum of 6 log CFU, as shown in Figure 1.

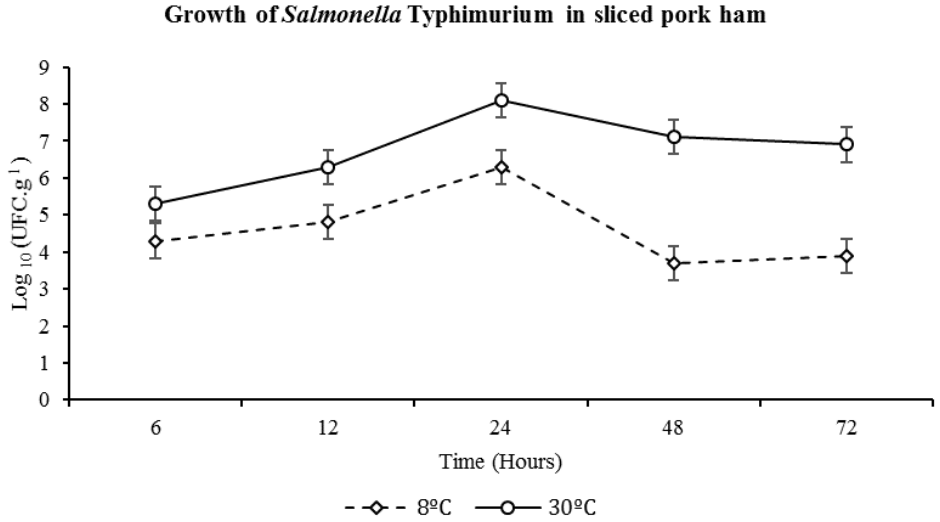

Figure 1: Growth kinetics of Salmonella Typhimurium in sliced pork ham at 8 ºC and 30 ºC. The treatment means showed statistically significant differences, as determined by the paired samples t-test (t = -4.6968, p = 0.0047, CI [95%] = -3.4048 to 0.8752).

The highest bacterial concentrations were recorded at a temperature of 30 ºC, which differed statistically from those achieved at 8 ºC (p>0.005, CI [95%] = -3.4048 to 0.8752). Furthermore, the peak bacterial growth at both temperatures occurred after 24 h of inoculation.

The growth of the microorganism in meat products at higher temperatures (30-40 ºC) is well described in the scientific literature. Nunes et al. (2021) [18] demonstrated pathogen growth at different temperatures (15 ºC to 42 ºC) in chicken meat, claiming that the growth of *Salmonella* spp. is accelerated as the temperature increases. Furthermore, Sabike et al. (2015) [14] pointed out that pathogen growth is significant with rising temperatures in ground meat, data that are in line with the results observed for ham in the present study.

However, there are discrepancies in the behavior of *Salmonella* spp. under refrigeration. Based in this, some studies report that there is no pathogen growth at 8 °C, as in the work of Sabike et al. 2015 [14] with ground meat, and the same observation was also made by Zaher and Fujikawa (2011) [15] in raw ground chicken. These findings, however, differ from those presented here, where significant concentrations of *Salmonella* spp. were recorded even under refrigeration.

It is worth noting that food security technologies and intrinsic and extrinsic food properties are factors that interfere with bacterial growth bacterial. Serra-Castelló et al. (2022) [19] reported a decline of the pathogen at 7ºC in dry cured ham treated with high pressure in different water activities (a_w). The authors noted that the main inhibiting factor was the storage temperature, but the food safety technologies used in the study and the varying a_w conditions favored the decline of the *Salmonella* spp. population.

Conversely, Szczawińska et al. (2014) [20] reported the survival capacity of *Salmonella* Enteritidis at 5 °C and 10 °C in cooked ham, in which the bacterial population remained at 3 log. However, despite no growth, the pathogen survived, which presents a risk to the consumer. A more recent study by Zhao et al. (2022) [21] reported the growth of the bacteria at 10°C in fresh pork. In addition, Noviyanti et al. (2024) [11] also confirmed the growth of the pathogen in these conditions in raw and cooked chicken.

Yet in this context, Mahgoub et al. (2024) [22] described in their study on smoked turkey in vacuum and modified atmosphere packaging that a temperature of 10 ºC is less effective and can favor the proliferation of *Salmonella* spp. However, the authors reported that lowering the temperature to 0ºC favors the decline but prolongs the survival of the pathogen, as viable cells were rescued after 120 days of cultivation under these conditions.

Furthermore, the authors reported that modifying the atmosphere promotes the survival of the pathogen in turkey. However, even with new security measures, proper cooking and temperature control are necessary to prevent the presence of this microorganism. The data of the present study, along with scientific literature, clearly demonstrate the ability of *Salmonella* spp. to adapt to temperature changes, posing a risk to the consumer. Therefore, new security measures should be investigated to control the pathogen growth in food [22]. These findings highlight the need for other conservation strategies and, above all, procedures that minimize the occurrence of contamination during processing.

The average generation time (G) at 8 and 30 °C were, respectively, 185.24 and 144.49 min. The speed specific (μ) was, respectively, 0.00041 and 0.00009 log/min. These results demonstrated that the growth rate of *S.* Typhimurium is greater at 30 °C, reaffirming the importance of temperature in the pathogen kinetics. Thus, although the temperature considerably influences the speed and bacterial cell multiplication dynamics in that food, it is important to emphasize that the pathogen survived in the substrate during the whole modeled period, which basically simulates the useful life of the product after it is removed from the packaging.

The kinetics of death were also evaluated, under a constant temperature of 60 ºC and 65 ºC. These data demonstrated the ideal treatment temperature and time required for inactivation of *S.* Typhimurium, offering insights for food safety in the industry. The thermal inactivation curves are shown in Figures 2 and 3.

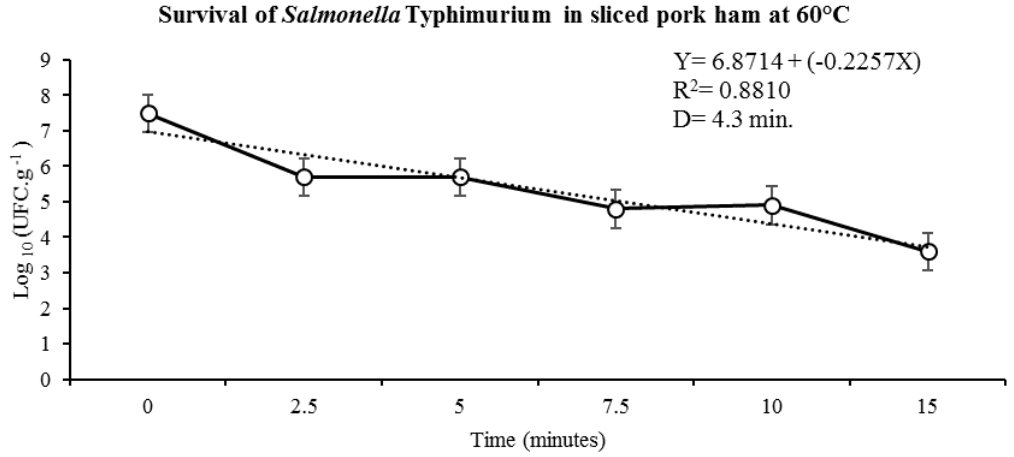

Figure 2. Thermal inactivation of Salmonella Typhimurium in raw and sliced ham at 60 ºC.

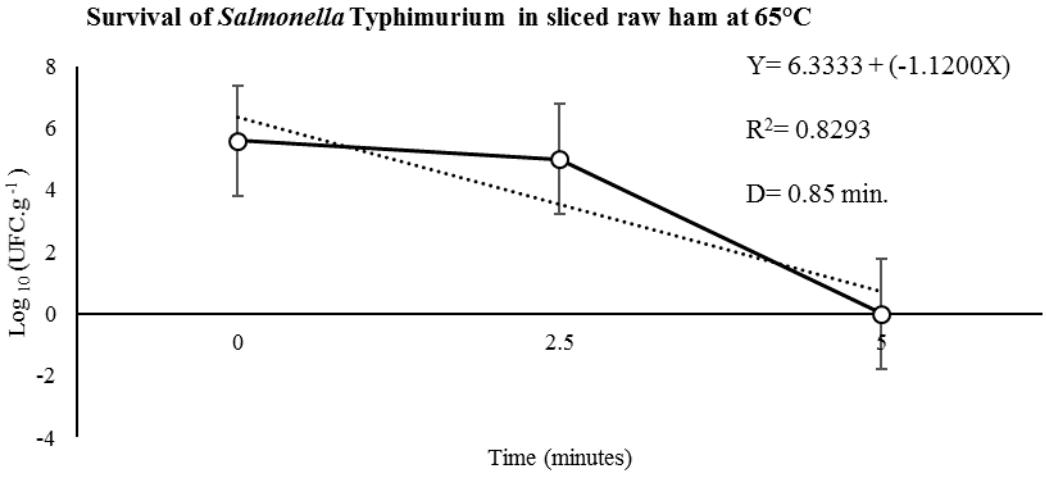

Figure 3. Thermal inactivation of Salmonella Typhimurium in raw and sliced ham at 65 ºC.

The results of this study demonstrated that the pathogen was capable of surviving at 60 °C for the 15 min of treatment (final concentration of 4 Log CFU.mL^{-1}), with a D value of 4.3 min, while at 65 ºC the strain was inactive in 5 min with a D value of 0.85 min. Therefore, temperatures above 60 º C are capable of inactivating *S.* Typhimurium in pork ham, and should be considered during food processing. Furthermore, these parameters are important for the food industry, which should rely on reliable metrics for product treatment, without altering its organoleptic features.

The data in the literature on thermal inactivation at these temperatures are variable, mainly due to the nutritional composition of the food. However, the present study is similar to the research by Ramirez-Hernandez et al. (2018) [23] on high - fat meat products, which underwent thermal treatment for 9 min. The authors report that *Salmonella* spp. was inactivated in 4 min at 65 ºC with a D value of 2.175 min. In contrast, the pathogen survived at 60 °C with a D value of 4.174 min, which reaffirms that temperatures greater than 60 ºC are necessary for the thermal inactivation of *Salmonella* spp. strains.

The nutritional composition of the food is also a crucial point in understanding thermal inactivation of *Salmonella* spp. Although the physical-chemical aspects of the food were not the objective of the present study, it is important mention that the food matrix interferes with inactivation kinetics, since according to Gurman et al. (2016) [24], the increase in fat content in pork food favors survival because fat serves as thermal protection. However, this effect can be inhibited at temperature above 60 ºC.

Thus, these findings point out the risk of consuming raw food and highlight the need to carefully control temperatures during food preparation. Heat must reach the entire product

(surface and internal portions) for the minimum time necessary to ensure the thermal inactivation of the bacteria and, therefore, food safety.

4. CONCLUSION

The highest bacterial concentrations occurred at 30º C, after 24 h of inoculation. However, the pathogen grew significantly at 8ºC, a temperature previously considered unviable for the growth of this microorganism. In addition, *S.* Typhimurium was resistant to 60ºC, but thermal treatment at 65°C inactivated the strain in 5 min. Thus, the present study presents the scientific community with a predictive model for the growth of *S.* Typhimurium in pork ham, and alert for its growth in conditions previously considered unfeasible. This highlights the need for new measures to prevent public health problems.

5. REFERENCES

1. Bosse (Née Danz) R, Müller A, Gibis M, et al. Recent advances in cured raw ham manufacture. Crit Rev Food Sci Nutr. 2018;58:610-30. doi: 10.1080/10408398.2016.1208634
2. Raimondi S, Luciani R, Sirangelo TM, et al. Microbiota of sliced cooked ham packaged in modified atmosphere throughout the shelf life. Int J Food Microbiol. 2019;289:200-8. doi: 10.1016/j.ijfoodmicro.2018.09.017
3. Albuquerque TG, Bragotto APA, Costa HS. Processed food: Nutrition, safety, and public health. Int J Environ Res Public Health. 2022;19:16410. doi: 10.3390/ijerph192416410
4. Spampinato G, Candeliere F, Amaretti A, et al. Microbiota survey of sliced cooked ham during the secondary shelf life. Front Microbiol. 2022;13:842390. doi: 10.3389/fmicb.2022.842390
5. Costa CMRD, Encarnação LSD, Souza EC, et al. Condição sanitária de presuntos fatiados comercializados em supermercados da cidade de Maceió, AL. Hig Aliment. 2017;31(274/275):76-80.
6. Wang S, Fu L, Chen G, et al. Multisite survey of bacterial contamination in ready-to-eat meat products throughout the cooking and selling processes in urban supermarket, Nanjing, China. Food Sci Nutr. 2020;8:2427-35. doi: 10.1002/fsn3.1532
7. Dos Santos DA, Nunes FL, Da Silva KO, et al. Effects of industrial slicing on the microbiological quality and safety of mozzarella cheese and ham. J Agric Food Res. 2024;16:101144. doi: 10.1016/j.jafr.2024.101144
8. Jaroni D. *Salmonella typhi*. In: Batt CA, Tortorello ML, editors. Encyclopedia of Food Microbiology (Second Edition). Oxford (US): Academic Press; 2014. p. 349-52. doi: 10.1016/B978-0-12-384730-0.00296-2
9. Ferrari RG, Rosario DKA, Cunha-Neto A, et al. Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a Meta-analysis. Appl Environ Microbiol. 2019;85:e00591-19. doi: 10.1128/AEM.00591-19
10. Lima JS, Sampaio APPO, Dufossé MCDS, et al. Growth kinetics of *Salmonella* Typhimurium and Listeria monocytogenes in buffalo milk under different processing and storage conditions. Ciênc Rural. 2021;51:e20200967. doi: 10.1590/0103-8478cr20200967
11. Noviyanti F, Mochida M, Kawasaki S. Predictive modeling of *Salmonella* spp. growth behavior in cooked and raw chicken samples: Real-time PCR quantification approach and model assessment in different handling scenarios. J Food Sci. 2024;89:2410-22. doi: 10.1111/1750-3841.17020
12. Brasil. Ministério da Saúde, Agência Nacional de Vigilância Sanitária. Resolução nº 216, de 15 de setembro de 2004. Dispõe sobre regulamento técnico de boas práticas para serviço de alimentação. Brasília (DF): Diário Oficial da União; 2004. Disponível em: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2004/res0216_15_09_2004.html.
13. Santos KPOD, Faria ACDSR, Silva PA, et al. *Salmonella* spp. como gente causal em doenças transmitidas por alimentos e sua importância na saúde pública: Revisão. Pubvet. 2020;14:1-9. doi: 10.31533/pubvet.v14n10a665.1-9
14. Sabike II, Fujikawa H, Edris AM. The growth kinetics of *Salmonella* enteritidis in raw ground beef. Biocontrol Sci. 2015;20:185-92. doi: 10.4265/bio.20.185
15. Zaher SM, Fujikawa H. Effect of native microflora on the growth kinetics of *Salmonella* enteritidis strain 04-137 in raw ground chicken. J Food Prot. 2011;74:735-42. doi: 10.4315/0362-028X.JFP-10-334
16. Brasil. Ministério da Saúde, Agência Nacional de Vigilância Sanitária. Instrução Normativa - IN nº 161, de 1º de julho de 2022. Estabelece padrões microbiológicos dos alimentos. Brasília (DF): Diário Oficial

da União; 6 jul 2022. Disponível em: https://www.in.gov.br/en/web/dou/-/instrucao-normativa-in-n-161-de-1-de-julho-de-2022-413366880.
17. Lemos WPC. Manual de laboratório de tecnologia química. Porto Alegre (RS): UFRGS; 1996.
18. Nunes ADS, De Oliveira AN, Costa JCCP, et al. Modelagem do crescimento de *Salmonella* spp. Em carne de frango armazenada em diferentes condições de temperatura / Modeling the growth of salmonella spp. In broiler meat stored at different temperature conditions. Braz J Dev. 2021;7:91163-75. doi: 10.34117/bjdv7n9-328
19. Serra-Castelló C, Desriac N, Jofré A, et al. Key Factors Determining the behavior of pathogens in dry-cured ham after high pressure processing. Appl Sci. 2022;12:12732. doi: 10.3390/app122412732
20. Szczawińska ME, Szczawiński J, Łobacz A. Effect of temperature on the growth kinetics of *Salmonella* Enteritidis in cooked ham. Bull Vet Inst Pulawy. 2014;58:47-56. doi: 10.2478/bvip-2014-0008
21. Zhao G, Yang T, Cheng H, et al. Establishment and application of a predictive growth kinetic model of *Salmonella* with the appearance of two other dominant background bacteria in fresh pork. Molecules. 2022;27:7673. doi: 10.3390/molecules27227673
22. Mahgoub SA, Qattan SYA, AlMalki F, et al. Impact of packaging atmosphere, oregano essential oil, and storage temperature on cold-adapted Salmonella Enteritidis and *Salmonella* Typhimurium on ready-to-eat smoked turkey. Poult Sci. 2024;103:103846. doi: 10.1016/j.psj.2024.103846
23. Ramirez-Hernandez A, Inestroza B, Parks A, et al. Thermal inactivation of *Salmonella* in high-fat rendering meat products. J Food Prot. 2018;81:54-8. doi: 10.4315/0362-028X.JFP-17-126
24. Gurman PM, Ross T, Holds GL, et al. Thermal inactivation of *Salmonella* spp. in pork burger patties. Int J Food Microbiol. 2016;219:12-21. doi: 10.1016/j.ijfoodmicro.2015.11.014