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# Bacterial growth in chicken breast fillet submitted to temperature abuse conditions

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**Abstract**

Given possible temperature variations in the cold chain during retail display of chilled food, this work evaluated the growth of *Salmonella choleraesuis* and *Staphylococcus aureus* inoculated in chicken breast fillet submitted to different temperature abuses. The bacterial growth was evaluated in Luria–Bertani broth and previously inoculated chicken breast fillet cooled for 12h and incubated at different temperatures (5, 20, and 25 °C) for 12 h and 5 °C for 12 days. The maximum growth rate and maximum growth were determined. The microorganisms grew at all studied temperatures, with a significantly lower growth at 5 °C compared with 20 and 25 °C. *S. choleraesuis* showed higher growth than *S. aureus* in both culture medium and chicken breast, and major maximum growth in culture medium than chicken breast, at all studied temperatures. *Salmonella* sp. and *S. aureus* were not detected in the control treatment maintained at 5 °C, and the thermotolerant bacteria remained within the standards allowed by Brazilian legislation when stored for 12 days. However, temperature abuse resulted in the vulnerability and spoilage of chicken breast fillet quality. The effects of temperature abuse caused by negligence on the microbial growth ($Y_{max}$) and growth rate ($\mu_{max}$) in chicken breast fillet, under industrial conditions was demonstrated.

**Keywords:** *Staphylococcus aureus*; *Salmonella choleraesuis*; thermotolerant bacteria; food safety; cold chain.

**Practical Application:** The kinetic and growth parameters of pathogenic microorganisms at abuse temperature was determinate.

## 1 Introduction

Efficient management of the food supply chain requires maintaining optimal product storage conditions from point of origin to point of consumption. According to good manufacturing practices, temperature is the main determinant of post-expiration dates, being the most important factor affecting food quality and safety (Taoukis, 2008; Taoukis et al., 2016).

Perishable products, such as fresh meat and especially cold chain products, may suffer temperature fluctuations and/or abuse, exceeding the safe storage limit of 5 °C (Laguerre et al., 2002; Nychas et al., 2008). These unexpected changes or cold chain temperatures may compromise food safety and quality due to the rapid growth of pathogenic bacteria such as *Salmonella choleraesuis* and *Staphylococcus aureus*, resulting in loss of consumer confidence and increased levels of food waste and economic losses (Franciosi et al., 2011; Gustavsson et al., 2011; Yehia et al., 2020).

Several studies have shown that temperature abuses occur at all stages of the cold chain and are related to many food products. Some products evaluated include fresh produce and its juice extracts (Huang et al., 2019), fruits and vegetables (Goedhals-Gerber et al., 2017), fresh meat, meat and vegetable preparations (Zubeldia et al., 2016), bagged salad (Brown et al., 2016), sliced ham (Derens-Bertheau et al., 2015), minced meat and processed fish (Lundén et al., 2014a) and ready-to-eat foods (Lundén et al., 2014b).

The cold chain is related to the quality of the final product by two different but complementary aspects: (i) microbiological contamination and the risk associated with human health; (ii) the organoleptic and sensory characteristics of the final product (Man, 2016; Mataragas et al., 2019). Therefore, microbial control and monitoring of the cold chain from production to final consumer is essential for the production of safe food with guaranteed shelf life (Kreyenschmidt et al., 2010). The temperature of household refrigerators and retail stores is considered a critical point in the supply chain (Limbo et al., 2010). The products displayed from supermarkets are handled by consumers and transported to their homes at a constant (25°C) high ambient temperature before returning to the freezer, so that microbial growth can occur (Karthikeyan et al., 2015).

In industries, temperature monitoring is usually performed by random measurements of the product core. In distribution, this aspect is mainly controlled by data loggers, which measure the ambient temperature. Due to their advantages, these monitoring systems are applied from production to retail (Koutsoumanis, 2001; Kreyenschmidt et al., 2010). However, cold chain interruptions can occur at the point of sale, on the retailer's path to the consumer and during home storage, and are not yet integrated into this monitoring concept (Limbo et al., 2010).

Poultry products have significant economic importance not only in Brazil but also around the word (Schuch et al.,

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2019; Schmidt et al., 2020; Auriema et al., 2019). But, only a few works of literature emphasize the growth of microorganisms in the chicken breast fillet with respect to the speed of growth in different temperature abuses caused by the negligence of some establishments due to the lack of temperature control of refrigerated exhibitors. foods. In this context, the present work evaluated the growth parameters (rate and maximum growth) of *Salmonella choleraesuis* and *Staphylococcus aureus* inoculated in chicken breast fillet and subjected to different temperature abuses (5, 20 and 25 °C) for 12 h.

## 2 Materials and methods

The chicken breast fillets were donated by a local agribusiness. This raw material was collected on the day of slaughter and cooled to 4 °C (Brastemp, Sao Paulo, Brazil) until the time of analysis. A correlation analysis between the microbiological growth in Luria–Bertani (LB) broth and chicken breast fillet subjected to temperature abuse was explored using *S. choleraesuis* (ATCC 10708) and *S. aureus* (ATCC 6538). The bacterial stock cultures were previously grown in LB broth (10 g/L tryptone; 5 g/L yeast extract; 5 g/L NaCl; Merck, USA). For bacterial growth, 0.1 µL of the stock cultures were transferred to LB broth and incubated at 37 °C for 24 h. Afterward, the inoculum was prepared by diluting the cell concentration in 0.1% peptone water to $10^2$ CFU/mL, and 1 mL of this dilution was transferred to test tubes containing 9 mL of LB broth. The cell count was enumerated by plating on plate count agar (PCA) medium (5 g/L tryptone; 2.5 g/L yeast extract; 1 g/L dextrose; 15 g/L agar).

To simulate negligence of the refrigeration in the store, the inoculum was maintained under refrigeration (5 °C) for 12 h to acclimatize to the cold before the refrigerator was switched off and the door opened to the environment (15 °C). After 2 h (lag phase), each inoculum was subjected to temperatures of 20 and 25 °C in an incubator for 10 h. In addition, after the abuse, a sample was conditioned again to refrigeration at 5 °C for 10 h. The growth kinetics in each condition were determined by plating and counting of the samples withdrawn from the inoculum each hour for 12 h, in triplicate.

*Salmonella choleraesuis* and *S. aureus* were inoculated separately into breast fillet pieces (8 $cm^3$ cubes) by immersion of the cubes in $10^2$ CFU/mL bacterial solution containing 0.1% peptone water, at room temperature (25 °C) for 10 min. The cubes were then removed from the broth, drained for 1 min. and incubated in polyvinyl chloride packages, hermetically sealed, and kept under refrigeration. Once reaching a stable temperature of 5 °C, the samples were conditioned in an incubator (Nova Ética, São Paulo, Brazil) at 5, 20, and 25 °C for 10 h. For the microbial count, a sample (around 10 g of chicken breast) was taken every hour and diluted in 90 mL of 0.1% peptone water, followed by plating on PCA medium, in the same manner as for the broth. This procedure was carried out in triplicate for each temperature and time evaluated.

The maximum growth rate ($\mu_{max}$) was determined according to the log of the growth variation ($dX$) as a function of time ($dt$), in the exponential phase (Equation 1):

$$\mu_{max} = \frac{dX}{dt} \quad (1)$$

The maximum growth ($Y_{max}$) was considered as the log of growth after incubation at different temperatures (5, 20, and 25 °C) for 12 h.

A control experiment (blank) was performed, in which samples of breast fillet without immersion in the bacterial inoculum were incubated at 5 °C for 12 days, with daily determinations of the counts of *S. aureus*, thermotolerant bacteria, and detection of *Salmonella* sp., in triplicate, based on a previous method (Silva et al., 2017).

The growth kinetic graphs with first order equations were constructed using LibreOffice software.

## 3 Results and discussion

Temperature variations can reduce the shelf life of perishable products, leading to a discrepancy between the final consumption date described on the food label and the legitimate conditions of food quality. The microbiological enumeration results of *S. choleraesuis* and *S. aureus* of the chicken breast fillets samples incubated at 5, 20, and 25 °C are shown in Figures 1 and 2.

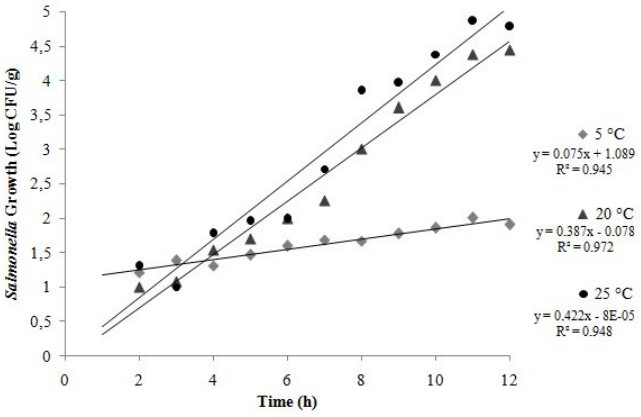


**Figure 1**. Growth kinetics of *Salmonella choleraesuis* at 5, 20, and 25 °C in chicken breast fillets.

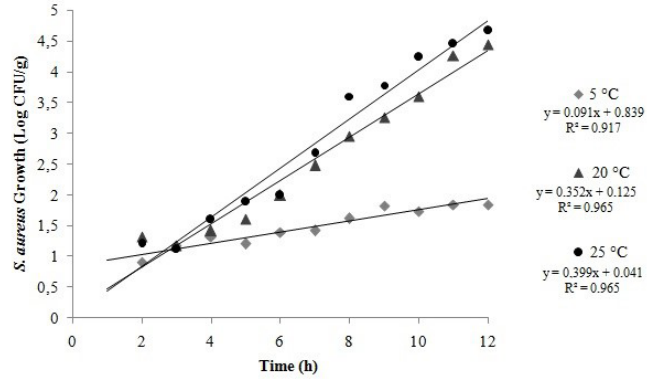


**Figure 2**. Growth kinetics of *Staphylococcus aureus* at 5, 20, and 25 °C in chicken breast fillets.

The temperature influenced the growth of *Salmonella choleraesuis*. The growth was constant until 2 h of incubation, characterizing the adaptation phase (lag phase) in the medium and the temperature conditions. Exposure to 25 °C resulted in an increase of approximately 4.78 log CFU/mL in 12 h, and a similar growth (4.44 log CFU/mL) was observed at 20 °C (12 h). Conversely, the samples exposed to 5 °C exhibited a count of 1.90 log CFU/mL (12 h).

*Staphylococcus aureus* exhibited a similar growth trend to *S. choleraesuis* under the different temperature conditions (Figure 2), so that it was almost constant until 2 h of incubation, characterizing the adaptation phase (lag). Temperature abuses of 20 and 25 °C resulted in a count of 4.43 and 4.66 log CFU/mL, respectively, at 12 h, which were much higher than 1.84 log CFU detected in the samples stored at 5 °C.

The heating that is caused by long periods of equipment shutdown, such as at night or on weekends, is a serious problem, providing favorable conditions for the development and reproduction of (often pathogenic) microorganisms, mainly on hot days, with high ambient temperature. This problem results in reduced shelf life, notably of perishable products. For this reason, cold chain management in food supply chains is receiving increasing attention from regulators, industry, and consumers (Ndraha et al., 2018).

According to the Resolution of the Collegiate Board of Directors (RDC) of the National Health Surveillance Agency (ANVISA) no. 216, of September 15, 2004 (Brasil, 2004), the equipment necessary for the consumer presentation or distribution must be appropriately sized, and the temperature monitored regularly. Semi-prepared and fully prepared foods must be cooled from 60 °C to 10 °C within 4 h or less, and stored under refrigeration at temperatures below 5 °C, or frozen at -18 °C. From that perspective, this study demonstrates the necessity of adequate low-temperature maintenance and control, as the temperature-abused product (above 20 °C) had a high microbiological count, representing a high safety risk to customers. According to Pereira et al. (2010), any failure in the cold chain (storage, preservation, distribution, transport, and handling of the products) can compromise the products' quality since the speeds of chemical, biochemical, and microbiological reactions are directly related to the temperature. In addition, the duration of the exposure to anomalous temperatures is equally decisive for refrigerated or frozen foodstuffs safety.

*Staphylococcus* are microorganisms commonly found among the microflora of raw poultry meat (Russell, 2008). Based on earlier research (Franco & Landgraf, 2008) between $10^5$ and $10^6$CFU of *S. aureus* per gram of food is necessary to form toxins at levels capable of causing intoxication. Considering this range in the context of the present study, the samples kept at 20 and 25°C for 12 h already pose a risk to consumer health, if the strain was enterotoxigenic.

For *S. choleraesuis* and *S. aureus* in LB broth and previously inoculated in chicken breast, submitted to different temperatures (5, 20, and 25 °C) after refrigeration (5 °C) for 12 h (Table 1), the temperature increase resulted in an increase in the $\mu_{max}$, with high rates for LB broth growth compared to those inoculated in chicken breast. *Salmonella choleraesuis* displayed a growth rate superior to *S. aureus* in both culture medium and chicken breast at 20 and 25 °C. At 5 °C, a low growth rate of *S. choleraesuis* was observed in both growing conditions. The $Y_{max}$ showed a similar behavior (Table 1), with high growth of *S. choleraesuis* in LB medium for all the temperatures evaluated. However, when inoculated in chicken breast samples, both bacteria had similar $Y_{max}$.

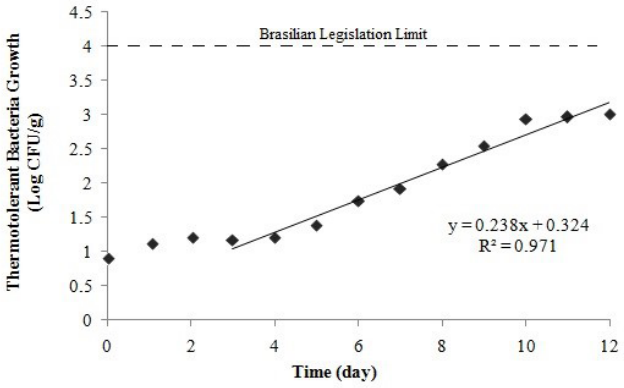


**Figure 3**. Growth kinetics of thermotolerant bacteria at 5 °C in chicken breast fillet.

**Table 1**. Maximum growth rate ($\mu_{max}$) and maximum growth ($Y_{max}$) of *S. choleraesuis* and *S. aureus* in LB medium and previously inoculated in chicken breast and subjected to temperature abuse.

| | Treatments | Growth parameters | Temperatures (°C) | | |
|---|---|---|---|---|---|
| | | | 5 | 20 | 25 |
| LB medium | ***S. choleraesuis*** | μmax (1/h) | 0.08 | 0.42 | 0.48 |
| | | Ymax (log CFU/mL) | 1.90 | 5.40 | 6.01 |
| | ***S. aureus*** | μmax (1/h) | 0.09 | 0.41 | 0.45 |
| | | Ymax (log CFU/mL) | 2.01 | 5.19 | 5.69 |
| Chicken breast | ***S. choleraesuis*** | μmax (1/h) | 0.07 | 0.34 | 0.38 |
| | | Ymax (log CFU/mL) | 1.90 | 4.45 | 4.71 |
| | ***S. aureus*** | μmax (1/h) | 0.09 | 0.31 | 0.34 |
| | | Ymax (log CFU/mL) | 1.84 | 4.44 | 4.67 |

$\mu_{max}$ = the specific growth rate (maximum growth rate); $Y_{max}$ = the maximum bacterial population.

*Salmonella* and *Staphylococcus* bacteria are heat tolerant but are susceptible to destruction when exposed to 55 °C for 1 h, or 60 °C for 15 to 20 min (Gama, 2001; Stewart, 2003). As mentioned by Franco & Landgraf (2008), bacterial growth is hampered by low temperatures. In the present work, microbial growth (albeit slow) was observed at low temperature (5 °C) after an initial temperature abuse of 15 °C for 2 h (Figures 1 and 2).

A control experiment performed with breast fillet without immersion in the bacterial inoculum did not detect counts of *S. aureus* and *Salmonella* sp. were absent. In analyzing the growth kinetics of the thermotolerant bacteria (Figure 3), delayed growth of both bacteria occurred in the control chicken breast fillets, incubated at 5 °C.

The chicken breast sample showed a shelf life of 12 days under ideal storage conditions, with a controlled temperature of 5 °C, consistent with the values established by the current Brazilian legislation, of 0 to 4 ± 1 °C for refrigerated products (Brasil, 1998; US Food and Drug Administration, 2017). However, the chicken breast samples that suffered temperature abuses (20 and 25 °C) should be consumed in the first 12 h. After this time, they would represent a risk to the consumers' health since the limit for a "safe contamination" is $10^4$ CFU/mL for thermotolerant bacteria (Brasil, 2001). In this light, it is essential to develop strategies for effective temperature control during the supply chain of fresh poultry products or development instruments that allow knowing the temperature values to which the food was exposed and predict its true useful shelf life.

Temperature is one of the most important factors affecting cellular metabolic reactions (Francis et al., 2012; Kou et al., 2014). It is also a critical factor in the survival and growth of pathogens in various food matrices (Huang et al., 2015; Luo et al., 2009; Luo et al., 2010; Sudarshana et al., 2008). Therefore, the proper maintenance of refrigeration during the transportation and storage of meat products is an extremely important practice for product quality and safety.

It should be noted that the low prevalence of all bacteria analyzed at time zero indicated a good process control during the processing stages, with good manufacturing practices by the slaughterhouse. According to the Brazilian Poultry Union (União Brasileira de Avicultura, 2015), the industrial sector has a great interest in the use of non-destructive and reliable techniques to validate thermal processes. A simple and inexpensive technological alternative to ensure the dynamic validity of perishable foods is the use of intelligent packaging containing a colorimetric indicator that can encourage the food producers to deliver products with safety guaranteed (Mehauden et al., 2007) since there is a constant pressure of the consumers and supervising systems for food safety.

## 4 Conclusion

Temperature is a determinant factor in food preservation, as observed in the microbiological results for *S. choleraesuis* and *S. aureus*, wherein chicken breast demonstrated high bacterial growth when exposed to temperature abuse (20 and 25 °C). *Salmonella choleraesuis* presented a high growth rate in LB broth and chicken breast fillet at 20 and 25 °C, and a low growth at 5 °C when compared with *S. aureus*. *Salmonella choleraesuis* also showed a high $Y_{max}$ in LB broth at all temperatures studied, and a similar $Y_{max}$ to *S. aureus* when inoculated in chicken breast. For the control sample preserved at favorable temperature conditions (5 °C), without temperature abuse during storage, the microbiological growth was stable during 12 days of analysis. Thus, this work demonstrates the importance of temperature control in chilled chicken breast fillet for the maintenance of quality and safety and can be extended to other meat products.

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