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Microbiological Effect of Cold Chain Temperature Simulation and Thawing Methods in Chicken Tenders

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Abstract

In this study, three storage temperatures-4 °C, -10 °C, and -20 °C for fridge (FD), freezer (F), and blast freezer (BF), respectively, as well as two thawing techniques for chicken tenders stored at two freezing temperatures (-10 °C and -20 °C) were used to examine the microbiological effects of temperature fluctuations in the cold chain. Three microbiological indicators, aerobic mesophilic bacteria (AMB), enterobacteriaceae (EB), and psychrotrophs (PSY), were counted using the Tempo® enumeration method. In the simulation study, the three microorganisms in FD had a rising tendency, but in the F and BF storages, AMB and EB displayed a steadier behavior, except for PSY, which displayed an increasing pattern. PSY were the ones with the greatest counts throughout all three storage types, although at BF temperatures, their numbers dropped before stabilizing. Contrarily, AMB and EB had the lowest numbers throughout all storages, although they showed an increased tendency in FD and a downward and steady trend in F and BF. The results regarding thawing methods revealed irregular patterns. In the case of BF and F storage, the initial (frozen) counts exhibited the highest numbers for AMB and PSY. Conversely, for the Fridge (24 H) thawing method, it showed the lowest counts for EB and PSY, but AMB had the lowest counts compared to Counter (6 H) thawing, where AMB counts were the highest. However, Counter (6 H) had lower counts for AMB but higher counts for EB, and Fridge (24 H) showing lower PSY counts, but higher for EB and AMB.

Keywords: blast freezer, microbiological indicators, temperature fluctuations

Resumen

En este estudio, se utilizaron tres temperaturas de almacenamiento - 4 °C, -10 °C y -20 °C para el frigorífico (FD), el congelador (F) y el congelador rápido (BF), respectivamente, también dos técnicas de descongelación para pechugas de pollo almacenadas a dos temperaturas de congelación (-10 °C y -20 °C), para examinar los efectos microbiológicos de las fluctuaciones de temperatura en la cadena de frío. Se enumeraron tres indicadores microbiológicos, bacterias mesófilas aerobias (AMB), enterobacteriáceas (EB) y psicrótrofas (PSY), utilizando el método de enumeración Tempo®. En la simulación, los tres microorganismos en FD tuvieron una tendencia ascendente, pero en los almacenamientos F y BF, AMB y EB mostraron un comportamiento más estable, exceptuando los PSY, ya que incrementaron. Los PSY presentaron mayores recuentos en los tres tipos de almacenamiento, aunque a temperaturas BF su número descendió antes de estabilizarse. Por el contrario, AMB y EB fueron las más bajas en todos los tipos de almacenamiento, aunque mostraron un aumento en FD y una tendencia descendente y constante en F y BF. Los métodos de descongelación revelaron patrones irregulares. En el caso de BF y F, los recuentos iniciales (congelados) mostraron los números más altos para AMB y PSY. Por el contrario, el método de descongelación Fridge (24 H), mostró los recuentos más bajos para EB y PSY, pero AMB tuvo los recuentos más bajos en comparación con la descongelación Counter (6 H), donde los recuentos de AMB fueron los más altos. Sin embargo, Counter (6 H) tuvo recuentos más bajos para AMB, pero más altos para EB, y Fridge (24 H) mostró recuentos más bajos para PSY, pero más altos para EB y AMB.

Palabras clave: congelador rápido, fluctuación de temperatura, indicadores microbiológicos

Introduction

Poultry production and consumption contribute significantly to the global food supply and economy. Poultry items, such as meat and eggs, have gained in popularity due to their excellent nutritional content, low cost, and convenience. According to the Food and Agriculture Organization of the United Nations (FAO), worldwide chicken production has risen dramatically in recent decades. It is projected to increase even more in the future. In 2021, global chicken meat output was estimated to be around 121.5 million tons (Food and Agriculture Organization of the United Nations [FAO], 2023)

In the other hand, raw meat and poultry are acknowledged as the predominant reservoirs for the transmission of *Salmonella* species to humans. It is estimated that around 40% of clinical cases of salmonellosis are attributable to the consumption of eggs and poultry products (Li et al., 2007). Also, due to the consumption trend, poultry production has increased, so has the number of foodborne illnesses related to poultry intake. According to a study conducted by the Centers for Disease Control and Prevention (CDC), poultry products were responsible for approximately 10% of foodborne illness outbreaks in the United States between 2009 and 2015 (Dewey-Mattia et al., 2018).

Chicken, being a perishable product, must be kept at certain storage temperatures, which can vary depending on how long users want to keep it before it can be consumed. The most common are refrigeration and freezing. The temperature that is usually considered is 4 °C for refrigeration, which is the one that is usually used for safety evaluations on chilled foods (Meng et al., 2019). On the other hand, freezing (-18 °C) is the safest way to preserve poultry meat (Pensiri et al., 2019).

Chicken should be kept at refrigeration temperature for no more than 2 days. According to the (U.S Food and Drug Administration [FDA], 2018), the refrigerator and freezer storage chart, the separated parts of the chicken can be refrigerated for 1-2 days, while frozen can be kept for 9 months. This is because many of the bacteria and fungi that can grow at refrigeration temperatures, grow slower and may not even grow at freezing temperatures. “The shelf life of food is extended by refrigeration because the metabolic processes of food-associated microorganisms are slowed by the

lowered temperature. Nonetheless, cold-adapted psychrotrophic food-poisoning and food-spoilage bacteria remain a concern because they possess cold-adapted proteins and membrane lipids that facilitate growth at low temperatures" (Russell, 2002).

There are several microorganisms to take into consideration that can grow in chicken meat at storage temperatures. The ones that are commonly found are, "*Pseudomonas spp.* (Gram negative, aerobe), Enterobacteriaceae (Gram negative, facultative anaerobe), and *Brochothrix thermosphacta* (Gram positive, facultative anaerobe), along with Lactic acid bacteria (Gram positive, aerotolerant anaerobe), and *Shewanella putrefaciens* (Gram negative, facultative anaerobe), representing the principal spoilage bacteria of fresh meats" (Loanna et al., 2017).

Mesophile aerobes comprise all bacteria, molds, and yeasts capable of growth within a temperature range of 35 °C ± 2 °C, under the specified conditions. To determine the number of aerobic bacteria, present in a food sample, the APC is utilized. However, a study states another method called Compact Dry TC. It is qualified as a rapid method to determine aerobic counts in a variety of raw meats (Kodaka et al., 2005).

Enterobacteriaceae organisms are notable contributors to severe infections, and many prominent members of this family are increasingly exhibiting resistance to currently available antimicrobial agents (Miranda et al., 2008). Is because of this reason that it is important to count these microorganisms, some methods are pour plates, spread plates, and the Most Probable Number (MPN) technique (Baylis, 2006).

Organisms that can grow under refrigerated temperatures are known as psychrotrophs and psychrophiles. However, the ones taken into consideration are the psychrotrophic bacterial counts, to determine them, the standard reference method of pouring plates is used. The plates are incubated at 7 °C for 10 days (Thomas, 1969).

In this study, the TEMPO® method is employed, aiming to provide a highly efficient approach for microorganism quantification while minimizing the potential for human error (Rajiv Kumar et al.,

2020). This MPN-based method is a fully automated enumeration system with the purpose of testing quality indicators in food (Grégory et al., 2005).

Food waste happens throughout all the process however, households are the ones who represent the highest contribution to this issue. Consumer handling is one of the main contributors to food spoilage and therefore, food waste. Despite efforts by processors using product portioning and recent advancements in packaging alternatives to extend shelf-life; there are still practices and product handling factors that continue to produce high amounts of food waste. Among intervention options that could reduce this burden, “Food (management) behaviors are usually considered, and they relate to many different aspects of the food product journey: planning, shopping, storage, preparation, and consumption of food. Food waste is an outcome of the way households deal with these different stages. The most cited consumer food management behaviors can be categorized into planning, (in-store) purchase, storage, preparation, serving, and leftover consumption practices” (Janssens et al., 2019).

Proper freezing and thawing practices are crucial for product quality and safety. Common thawing methods include kitchen counter, refrigerator, warm water, microwave, and tap water, but leaving frozen food at room temperature is not recommended due to bacterial activation. The Food Safety and Inspection Service (FSIS) recommends thawing chicken in the refrigerator as the safest method. This study investigates how temperature changes in storage and different thawing methods impact the microbiological aspects of chicken tenders stored at varying freezing temperatures (Benli, 2016; United States Department of Agriculture [USDA], 2019).

Materials and Methods

Study Location

Located in Lubbock, Texas, the research study was carried out at the facilities of Texas Tech University's Department of Animal and Food Science. Microbiological studies were conducted at the Experimental Sciences Building in the ICFIE Laboratories, and samples were stored in the Gordon W. Davis Meat Science Laboratory, a separate facility.

Microbiological Analysis

A microbiological study to evaluate the shelf-life of chicken tenders in eleven sampling points in the distribution value-chain was conducted. Freshly processed chicken tenders were procured from a commercial processor in the Southeast region of the US and shipped under refrigerated conditions to the ICFIE Laboratories. For Aerobic Counts (AC), the Association of Official Agricultural Chemists (AOAC) 121.204 was used, where TEMPO cards were incubated for 22-28 h at 35 ± 1 °C. For *Enterobacteriaceae* (EB), the AOAC 050801 was used, where TEMPO cards were incubated for 22-28 h at 35 ± 1 °C. Finally, for psychrotrophs (PSY), TEMPO cards were incubated for 6 days at 7 ± 1 °C. Added to this, the same enumeration process was applied for the second experiment with an initial count and 2 different thawing methods. At the same time, the ranges of detection for each dilution used were taken into consideration (Table 1).

The initial dilutions that were used are:

AC → 1/4 → 3 mL in Vial → 1mL of Sample in Vial

EB → 1/40 → 3.9 mL in Vial → 100 µL of Sample in Vial

PSY → 1/40 → 3.9 mL in Vial → 100 µL of Sample in Vial

PSY → 1/40,000 → 3 mL in Vial → 1 mL of Sample from the test tube of dilution 10^4 in Vial

Table 1

The ranges of detection for each dilution

Dilution	Range of detection
1/4	1 to 4.9x10^3 cfu/g or mL
1/40	10 to 4.9x10^4 cfu/g or mL
1/40,000	10^4 to 4.9x10^7 cfu/g or mL

Experimental Design

A completely randomized design was made with measures repeated over time, performing five replicates for each treatment. The analysis was carried out with the statistical program R Studio, making an ANOVA and a non-parametric analysis called the Pairwise Wilcoxon Test. Each one of the treatments, these being the chicken at refrigeration, freezing and blast freezing storage, had five replicates and the samplings were carried out in two stages, 6 within a simulation in the first five days and five sampling points on the day 8, 11, 14, 17 and 20 (Table 2). A second experiment evaluating three treatments consisting of the initial counts, as well as two different thawing methods: Fridge (24H) and Counter (6H) (Table 3).

Table 2

Cold chain temperature simulation

Sampling Point Number	Sampling Point	Description
1	Sample reception	Chicken tenders were received at refrigeration temperature
2	After 2 days of transportation	All samples were left for 48 hours inside a fridge at 7 °C and others in a freezer at -10 °C. Simulating the transportation to the storage.
3	After 1 hour of transition to warehouse	All samples were left for 1 hour at room temperature simulating the transition from the truck to the warehouse.
4	After 23 hours of storage at warehouse	All samples were left for 23 hours at their respective storage temperature being these at 4 °C for the fridge, -10 °C for the freezer and -20 °C for the blast freezer simulating the storage at a warehouse before the retail.
5	After 20 hours of retail	All samples were left for 20 hours at a retail display at temperatures from 4 °C – 10 °C

Sampling Point Number	Sampling Point	Description
6	Temperature abuse	All samples were left at room temperature for 4 hours simulating all the processes of the consumer routine for buying their groceries until they store them at home.
7	Day 8	It was used as a mapping to monitor the growth curve of microorganisms at the same 3 temperatures used for storage (4 °C, -10 °C, and -20 °C).
8	Day 11	It was used as a mapping to monitor the growth curve of microorganisms at the same 3 temperatures used for storage (4 °C, -10 °C, and -20 °C).
9	Day 14	It was used as a mapping to monitor the growth curve of microorganisms at the same 3 temperatures used for storage (4 °C, -10 °C, and -20 °C).
10	Day 17	It was used as a mapping to monitor the growth curve of microorganisms at the same 3 temperatures used for storage (4 °C, -10 °C, and -20 °C).
11	Day 20	It was used as a mapping to monitor the growth curve of microorganisms at the same 3 temperatures used for storage (4 °C, -10 °C, and -20 °C).

Table 3

Thawing methods

Type of Storage	Thawing Method	Description
Freezer/Blast Freezer	Initial	Chicken tenders were analyzed at storage conditions being these at Freezing and blast freezing temperatures, -10 °C and -20 °C respectively.
	Fridge (24 h)	Chicken tenders were analyzed after 24 h at refrigeration temperatures (4 °C)
	Counter (6 h)	Chicken tenders were analyzed after 6 h at a counter at room temperature.

Results and Discussion

Cold Chain Temperature Simulation

Aerobic Mesophilic Bacteria

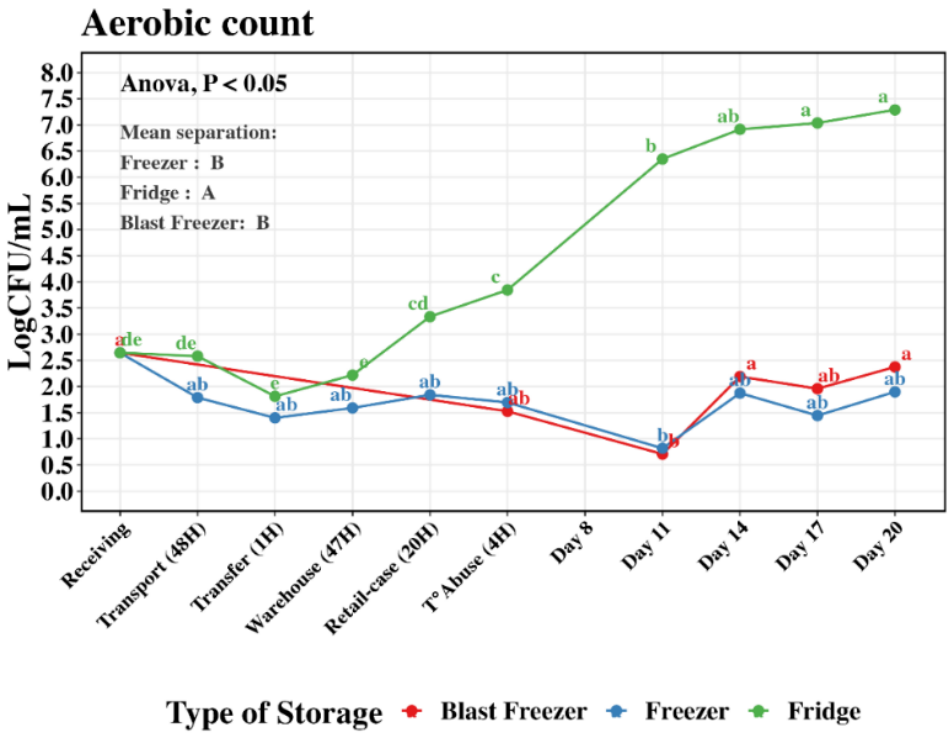
Figure 1 shows a growth curve of aerobic mesophilic bacteria during each sampling point, with the x-axis denoting the sampling points and the y-axis representing the Log CFU/mL. An analysis of variance (ANOVA) revealed a statistically significant difference ($P < 0.05$) among the treatments, specifically between the fridge and the blast freezer, as well as between the fridge and the freezer. These treatments correspond to the different types of storage under investigation. Furthermore, the blast freezer and freezer treatments exhibited a stable behavior pattern, characterized by the lowest point observed on day 11 and the highest point recorded on the receiving day, day 14, and day 20. However, there were no significant differences ($P > 0.05$) in the means observed between these sampling points, as confirmed by the separation of means analysis. Importantly, the stable behavior persisted during the receiving and transportation days. During these periods of time, the chicken tenders were stored at the same temperature conditions (4 °C, -10 °C and -20 °C). As well, on days 11, 14, 17, and 20 they were stored at the same temperatures of storage, therefore, no significant differences ($P > 0.05$) were observed among the sampling points.

The behavior of these bacteria at the different temperatures studied, 4 °C, -10 °C and -20 °C for fridge, freezer, and blast freezer respectively, may be due to the fact that the optimum temperatures for the growth of aerobic mesophilic bacteria are 32 °C to 37 °C, however, it has temperature limits from 5 °C to 46 °C (Balcázar, 2019; Pinzon Fernandez, 2006). This could be reinforced by the results obtained in a study by Russel (1995), where he found that freezing broiler carcasses at a temperature of -7.8 °C significantly lowered the populations of mesophilic aerobic bacteria as well as all temperatures below that, mentioned above (-10 °C and -20 °C). Unlike the carcasses at a temperature of 0 °C and -3.7 °C in which no significant differences were found reducing the loads of these same bacteria. Also, it was found that at a refrigeration temperature of 3 °C the

aerobic mesophilic bacteria increase significantly, like what was observed in the growth curves in the 3 types of storage.

Figure 1

Aerobic Mesophilic Bacteria growth curve of 3 types of storage for the cold chain temperature simulation



Note. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Receiving, Transport (48H), Transfer (1H), Warehouse (47H), Retail-case (20H), T° Abuse (4H), Day 8, Day 11, Day 14, Day 17, and Day 20 represents the mean of five replicates. $^{a\text{-}e}$: Different letters in each line indicate significant differences ($P < 0.05$) between treatments and sampling points.

Enterobacteriaceae

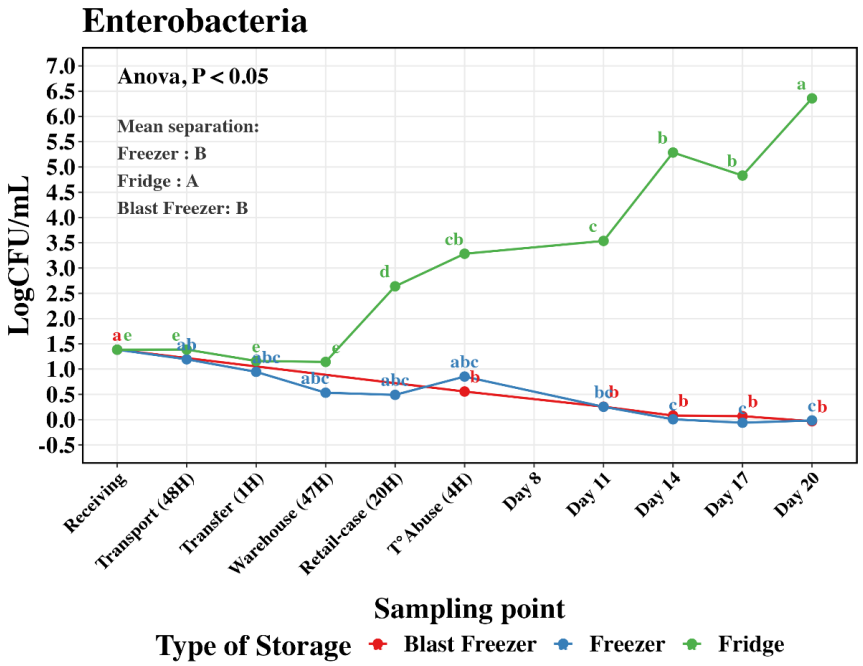
The growth curve of Enterobacteriaceae at each sampling point is shown in Figure 2. The x-axis corresponds to the sampling points, while the y-axis represents the Log CFU/mL. An ANOVA analysis with a significance level of $P < 0.05$ revealed significant differences between treatments, specifically the fridge treatment compared to the blast freezer and freezer treatments. These treatments represent the different types of storage investigated. Moreover, a stable behavior pattern

was observed in both the blast freezer and freezer treatments. The blast freezer treatment exhibited the highest point on the day of receipt, and no significant differences were observed in the remaining sampling points. Similarly, the freezer treatment displayed stable behavior throughout the study, unlike the fridge treatment, which demonstrated an increasing trend. The fridge treatment showed stability on the day of receiving, during transport, transfer, and warehouse. Additionally, similar behavior was observed on the 11th and 14th days and a peak on the 20th day is this the highest point of the curve.

This decreasing behavior presented in the Enterobacteriaceae could be due to the fact that the conditions at these temperatures do not allow population growth, since this type of bacteria develops optimally at temperatures of 30 °C to 37 °C. However, they have the ability to grow at minimum temperatures of 5 °C and maximum temperatures of 44 °C, which may be the explanation for the growth of enterobacteria at the Fridge storage temperature of 4 °C. Likewise, it may be due to this temperature range where Enterobacteriaceae grow, that no prevalence or growth was seen at lower temperatures such as for Freezer and Blast Freezer at -10 °C and -20 °C respectively. (Balcázar, 2019; Rogers et al., 2016). However, the range of storage temperatures in the fridge can be correlated with the findings of Halkman and Halkman (2014), where they agree with a lower range of growth temperatures, indicating that certain psychrotrophic Enterobacteriaceae can grow at 0 °C. In a study conducted by Nazarowec-White and Farber (1997), a behavior can be observed that could explain what happened with the growth curve of Enterobacteriaceae, where they evaluated the survival of *Enterobacter sakazakii* at different storage temperatures (4 °C, 10 °C and 23 °C). At temperatures of 10 °C and 23 °C, growth was observed in a time of 4.98 h and 40 min, respectively. On the other hand, this same microorganism had no opportunity to grow at 4 °C, and shortly after it began to decrease.

Figure 2

Enterobacteriaceae growth curve of 3 types of storage for the cold chain temperature simulation



Note. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Receiving, Transport (48H), Transfer (1H), Warehouse (47H), Retail-case (20H), T° Abuse (4H), Day 8, Day 11, Day 14, Day 17, and Day 20 represents the mean of five replicates. $^{a\text{-}e}$: Different letters in each line indicate significant differences ($P < 0.05$) between treatments and sampling points.

Psychrotrophs

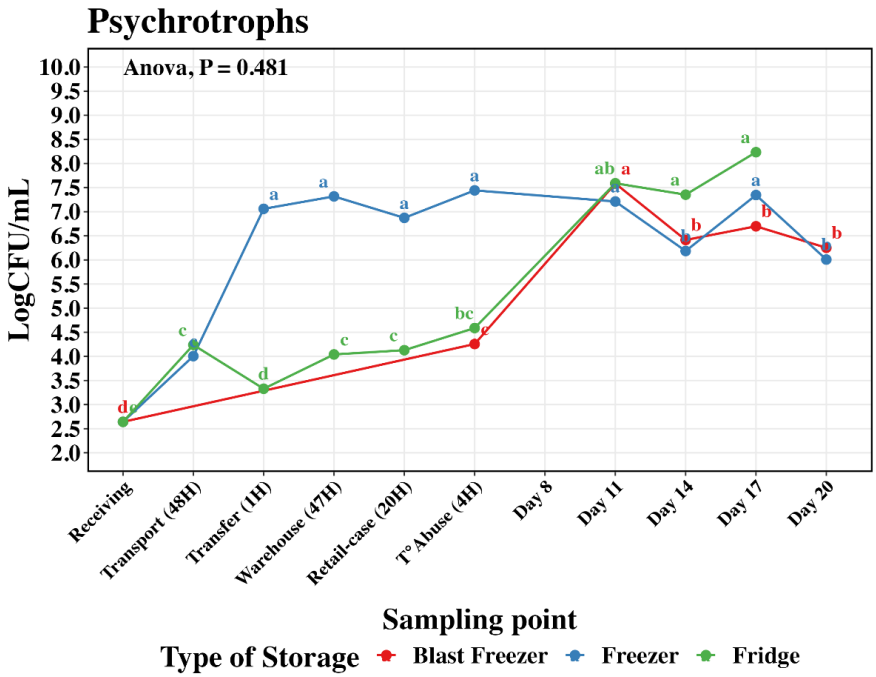
The growth curve of psychrotrophs at each sampling point is displayed in the Figure 3. The x-axis corresponds to the sampling points, while the y-axis represents the Log CFU/mL. An ANOVA analysis with a significance level of $P = 0.481$ showed that there were no significant differences among treatments, which included the blast freezer, freezer, and fridge. In all three treatments, irregular behavior can be observed. However, it is worth noting that for the blast freezer treatment, an increase was observed up to day 11, which was the highest point on the curve. Subsequently, the curve remained stable on days 14, 17, and 20, suggesting no significant differences between these sampling points. For the freezer treatment, there was a growth peak up to the transfer point, and it remained

stable until day 11, followed by fluctuations on days 14, 17, and 20. Finally, in the fridge treatment, an upward trend can be observed until day 11, after which the curve remained stable on days 14 and 17.

Berry and Foegeding (1997) indicate that psychrotrophs are microorganisms known for their ability to grow at temperatures as low as 5 °C. This phenomenon could explain the upward trend observed in the Fridge treatment. However, psychrotrophs thrive best within the temperature range of 25 °C to 30 °C. It is this specific temperature range that causes the increasing pattern in all types of storage. Importantly, the Freezer and Blast Freezer treatments show a noticeable turning point, after which the number of microbes stabilizes before decreasing. This drop in population can be attributed to the fact that the temperatures in these types of storage are lower (-10 °C to -20 °C) than the minimum and maximum range suitable for psychrotrophs (5 °C to 42 °C) (Pérez Álvarez et al., 2015). Although the temperature ranges of fridge, freezer and Blast Freezer do not agree with the optimal ones for the growth of psychrotrophs. In the study conducted by Walter de Santana et al. (2020), it was found that *pseudomonas*, being a psychrotrophs microorganism, have the ability to proliferate at refrigeration temperatures (0 – 4 °C) regardless of their optimal range of growth. Since that microorganisms grow at lower temperatures (0 – 15 °C) are categorized as psychrophiles, they can survive at temperatures below 0 °C (Berry & Foegeding, 1997).

Figure 3

Psychrotrophs growth curve of 3 types of storage for the cold chain temperature simulation



Note. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Receiving, Transport (48H), Transfer (1H), Warehouse (47H), Retail-case (20H), T° Abuse (4H), Day 8, Day 11, Day 14, Day 17, and Day 20 represents the mean of five replicates. [a-d]: Different letters in each line indicate significant differences ($P < 0.05$) between treatments and sampling points.

Fridge

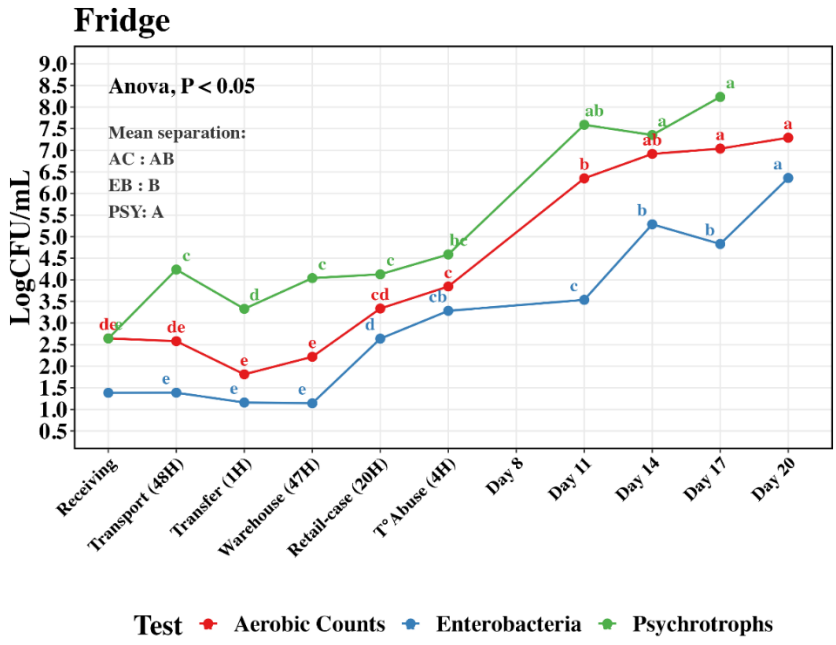
In Figure 4 the behavior of the three microorganisms (Aerobic Mesophilic Bacteria, Enterobacteriaceae, and psychrotrophs) is shown within the Fridge treatment where the x-axis corresponds to the sampling points, while the y-axis represents the Log CFU/mL. An ANOVA analysis with a significance level of $P < 0.05$ was conducted, demonstrating the mean separation between microorganisms. For all three microorganisms studied, an ascending trend is observed throughout the sampling points, resulting in stability during the receiving, transport, transfer, and warehouse stages for Enterobacteriaceae and aerobic mesophilic bacteria. In contrast, psychrotrophs exhibited fluctuation at these four mentioned sampling points. Notably, both aerobic mesophilic bacteria and psychrotrophs showed stability on days 11, 14, 17, and 20. Furthermore, it can be concluded that the

microbiological growth was statistically higher in psychrotrophs compared to aerobic mesophilic bacteria, followed by Enterobacteriaceae. Significantly, there are differences ($P < 0.05$) between psychrotrophs and Enterobacteriaceae.

The behavior of the three microorganisms evaluated (Aerobic Mesophilic Bacteria, Enterobacteriaceae, and psychrotrophs) was ascending; this could be due to the fact that at a storage temperature of 4 °C the aforementioned microorganisms have the capacity to develop (Balcázar, 2019; Halkman & Halkman, 2014; Walter de Santana et al., 2020). Additionally, in study by Paseto Fernandes et al. (2014), where they evaluated in lamb meat a growth curve of several microorganisms (*Salmonella*, coagulase-positive staphylococci, aerobic and anaerobic psychrotrophic bacteria, and lactic acid bacteria) stored at 1 °C in a modified atmosphere package (MAP), it was observed that there was stability in the first 7 days and then an increase until day 21, similar to the behavior shown in Figure 4 for the simulation of the three indicators at Fridge storage without any barrier from the environment.

Figure 4

Growth curve of 3 indicators of fridge storage for the cold chain temperature simulation



Note. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Receiving, Transport (48H), Transfer (1H), Warehouse (47H), Retail-case (20H), T° Abuse (4H), Day 8, Day 11, Day 14, Day 17, and Day 20 represents the mean of five replicates. $^{a\text{-}e}$: Different letters in each line indicate significant differences ($P < 0.05$) between treatments and sampling points.

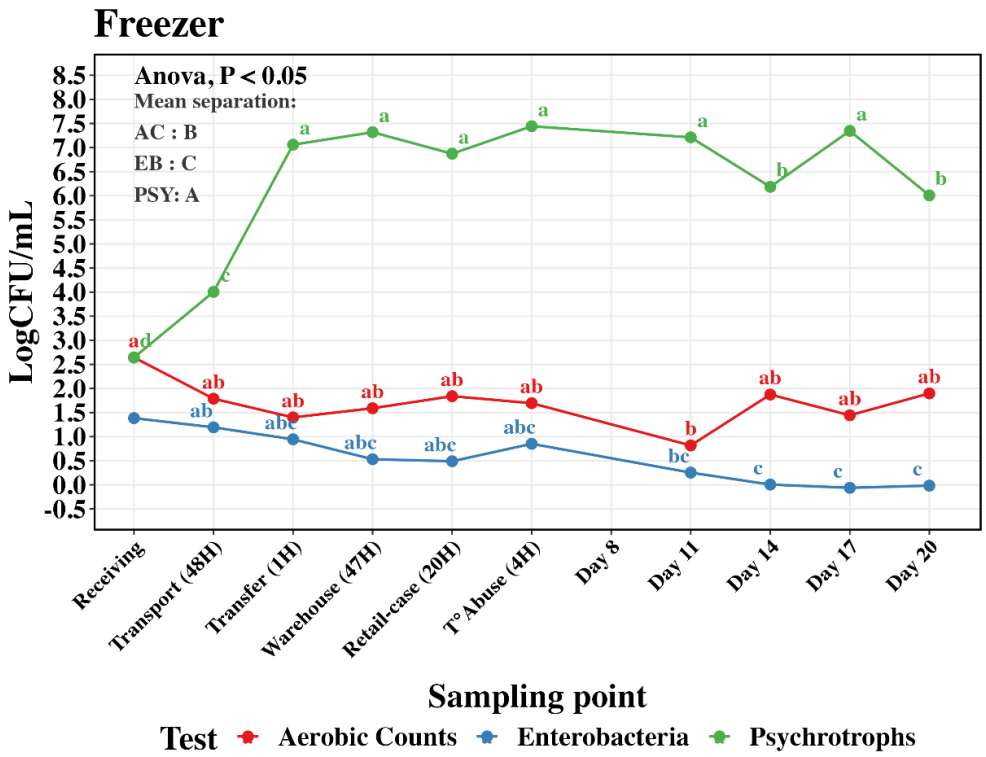
Freezer

In Figure 5 the behavior of the three microorganisms (Aerobic Mesophilic Bacteria, Enterobacteriaceae, and psychrotrophs) is shown within the freezer treatment where the x-axis corresponds to the sampling points, while the y-axis represents the Log CFU/mL. An ANOVA analysis with a significance level of $P < 0.05$ and a mean separation between the three microorganisms. A stable trend is evident in the behavior of the three microorganisms, except for psychrotrophs, which exhibit an upward trajectory in the first two sampling points, specifically during the stages of transport and transfer. Importantly, statistical analysis reveals significant differences between the three microorganisms based on mean separation, with psychrotrophs displaying the highest counts, followed by aerobic mesophilic bacteria, and finally enterobacteriaceae.

The behavior is evident by observing a higher population of psychrotrophic bacteria; it could have been given by the same reason explained above, in which these bacteria have the ability to withstand low temperatures even below 0 °C (Berry & Foegeding, 1997). In the case of Enterobacteriaceae and aerobic mesophilic bacteria, these have a higher optimal temperature range (30 – 37 °C). Given the above-mentioned information, this is likely why they could not grow at freezing temperatures (18 °C) (Balcázar, 2019; Rogers et al., 2016). This can be better explained by a study conducted by Georgsson et al. (2006), where they evaluated the effect of freezing on microorganisms in chicken meat, in which, it was observed that microorganisms such as *Campylobacter spp.* and *C. jejuni* reduced their population after the chickens were frozen, agreeing with those found in the present study, where a decreasing trend is seen for this group of bacteria, the Enterobacteriaceae. On the other hand, AMB were affected by low temperatures; these results agree with those obtained by El-Shibiny et al. (2009), where they found in their study that Campylobacter jejuni and Campylobacter coli bacteria reduced their counts when chicken skin was frozen at -20 °C. It was shown that these aerobic mesophilic bacteria were reduced at a rate of 0.34 log10 CFU/cm2/day when maintained at the aforementioned temperature.

Figure 5

Growth curve of 3 indicators of freezer storage for the cold chain temperature simulation



Note. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Receiving, Transport (48H), Transfer (1H), Warehouse (47H), Retail-case (20H), T° Abuse (4H), Day 8, Day 11, Day 14, Day 17, and Day 20 represents the mean of five replicates. $^{a-c}$: Different letters in each line indicate significant differences ($P < 0.05$) between treatments and sampling points.

Blast Freezer

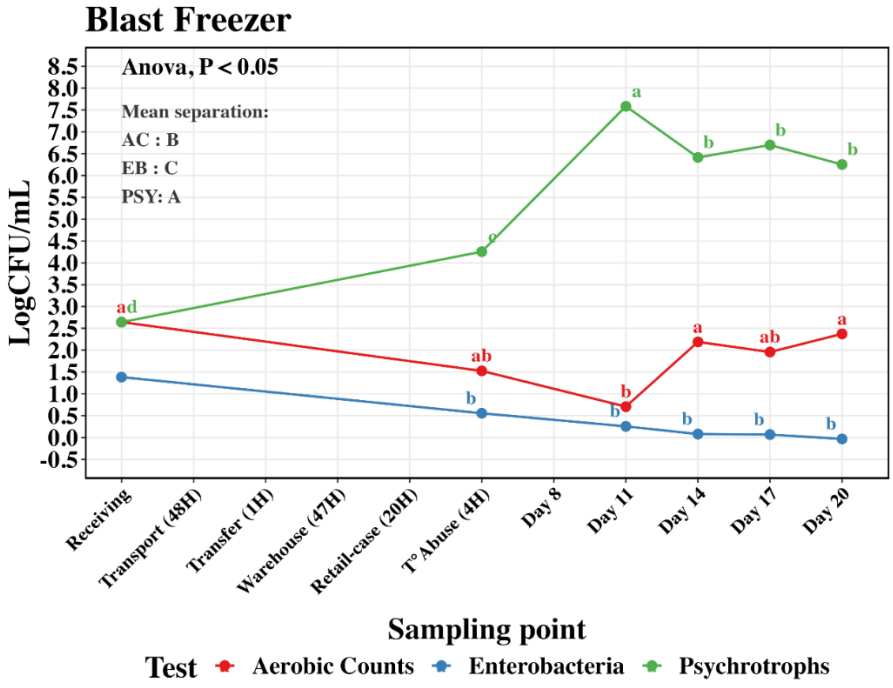
In Figure 6 the behavior of the three microorganisms (Aerobic Mesophilic Bacteria, Enterobacteriaceae, and psychrotrophs) is shown within the blast freezer treatment where the x-axis corresponds to the sampling points, while the y-axis represents the Log CFU/mL. An ANOVA analysis with a significance level of $P < 0.05$ and a mean separation between the three microorganisms. Significant variations are evident among the three microorganisms, whereby psychrotrophs demonstrate the highest population levels, followed by aerobic mesophilic bacteria, and finally Enterobacteriaceae. However, it is important to note that the growth curves of Enterobacteriaceae and aerobic mesophilic bacteria exhibit a stable pattern, in contrast to the growth curve of

psychrotrophs, which displays an ascending trend during the initial three sampling points encompassing receiving, temperature abuse, and day 11, followed by a subsequent decline and stabilization on days 14, 17, and 20.

It can be highlighted by the behavior shown that those with the lowest counts were the Enterobacteriaceae, followed by the aerobic mesophilic bacteria, and finally, with the highest count, the psychrotrophs. This agrees with a study conducted by Olgunoglu (2010), where they evaluated the effect of air blast freezing on the microbial load (aerobic plate count, psychrotrophic flora count, coliforms, and *E. coli*, among others) in fish fillets. They found in the study that blast freezing reduces microbial loads since the application of very low temperatures can damage the cell membrane of bacteria and their DNA. At the same time, they found that psychrotrophic bacteria had the highest loads, while aerobic mesophilic bacteria had the lowest loads. On the other hand, the growth curve observed for psychrotrophs in Figure 6, align with the resistance of the microorganisms to different temperatures, such as psychrotrophs that can grow at temperatures below 7 °C. Therefore, this may be one of the reasons why they had the highest populations (Suhren, 2020).

Figure 6

Growth curve of 3 indicators of blast freezer storage for the cold chain temperature simulation



Note. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Receiving, Transport (48H), Transfer (1H), Warehouse (47H), Retail-case (20H), T° Abuse (4H), Day 8, Day 11, Day 14, Day 17, and Day 20 represents the mean of five replicates. [a-d]: Different letters in each line indicate significant differences ($P < 0.05$) between treatments and sampling points.

Thawing Methods

Aerobic Mesophilic Bacteria

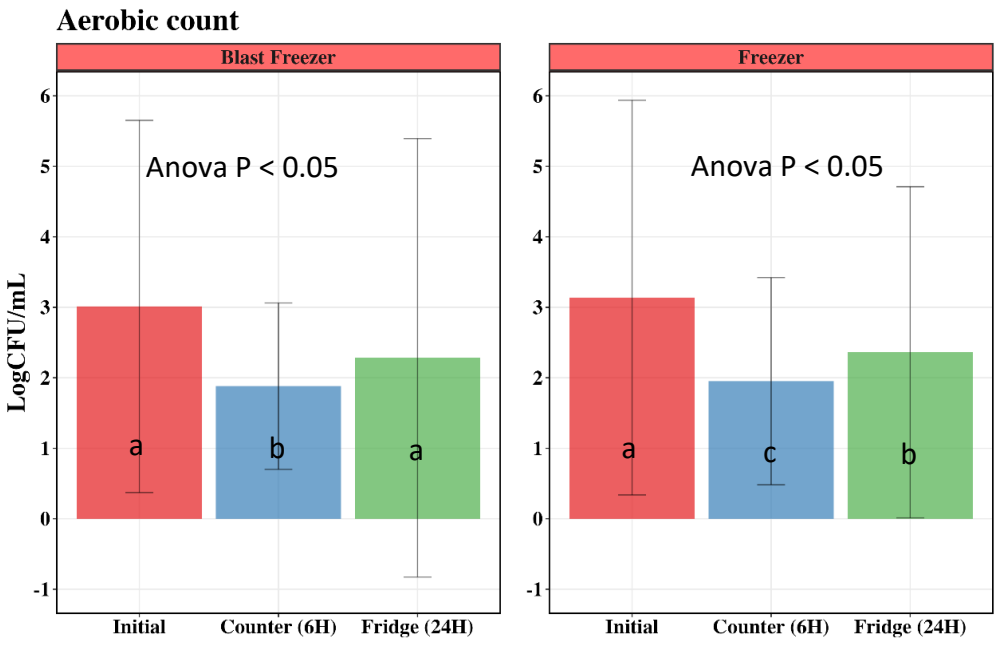
The provided figure illustrates the behavior of aerobic mesophilic bacteria in relation to various thawing methods, denoted on the X-axis as Initial, Counter (6 H). The Y-axis represents the logarithmic colony-forming units per milliliter (Log CFU/mL), serving as a measure of aerobic microorganism growth. The figure effectively demonstrates the growth patterns observed after thawing from two distinct storage types (Blast Freezer and Freezer). Furthermore, the figure includes a clear separation of means between the thawing methods and an accompanying error line. The analysis of the blast freezer storage type reveals significant differences between treatments, as indicated by the separation of means at a significance level of $P < 0.05$. The initial treatment exhibits

higher microorganism counts compared to the counter (6H) and fridge (24H) treatments, where no significant differences are observed between the latter two. Likewise, in the case of freezer storage, a similar pattern is observed. All treatments display significant differences, as demonstrated by the separation of means at a significance level of $P < 0.05$. The initial treatment yields the highest microorganism counts, followed by the fridge (24H) treatment, and the counter (6H) treatment exhibits the lowest count (Figure 7).

These behaviors shown in Figure 7 might be clarified through an examination of the sampling methodology employed. As is commonly understood, consumers typically extract only the chicken from the residual water in the bag or container when thawing chicken parts. This same method was utilized when obtaining samples of the chicken tenders after the designated time (6 H Counter and 24 H Fridge). Because the tenders were treated with buffered peptone water in a separate bag, the melted ice could have influenced the actual microbial count. A similar pattern was observed in a study conducted by Kim et al. (2022), in which they determined that the thawing process, leading to drip loss and exudates from the defrosted chicken breasts, could disrupt the meat structure and potentially boost the population of aerobic mesophilic bacteria. This is due to the provision of optimal resources and conditions for bacterial proliferation. Notably, the exudates from the thawed chicken tenders and the water present in the bag were not considered during the sampling procedure. The amplitude of the error lines could be due to the number of replicates increasing the variability in the data. On the other hand, the human error during the project development and the aforementioned reason for the behavior could have an impact.

Figure 7

Aerobic mesophilic bacteria growth behavior in relation to blast freezer and freezer storages for the thawing methods



Note. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Initial, Counter (6H), and Fridge (24H) represents the mean of five replicates. [a-c]: Different letters in each line indicate significant differences ($P < 0.05$) between treatments. Each error line in each column represents the variability in the recollected data.

Enterobacteriaceae

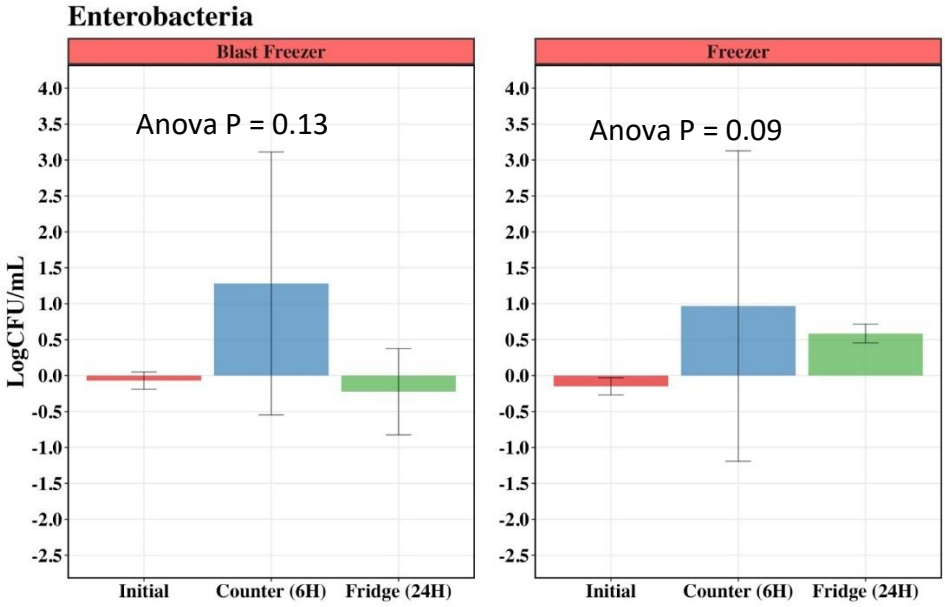
The Figure 8 illustrates the behavior of enterobacteriaceae in relation to various thawing methods, denoted on the X-axis as Initial, Counter (6 H), Fridge (24 H), and Fridge (24H). The Y-axis represents the logarithmic colony-forming units per milliliter (Log CFU/mL), serving as a measure of enterobacteriaceae microorganism growth. The figure effectively demonstrates the growth patterns observed after thawing from two distinct storage types (Blast Freezer and Freezer). Furthermore, the figure includes a clear separation of means between the thawing methods and an accompanying error line. Regarding the blast freezer storage type, the analysis reveals an ascending trend in enterobacteria counts as thawing progresses in the counter treatment (6H). Conversely, a decline is observed in the fridge treatment (24H) compared to the initial count. However, it is worth noting that

even though arithmetically and visually the results are separated, no statistically significant differences were detected between treatments at a significance level of P = 0.13. Similarly, in the case of freezer storage type, the initial treatment displays the lowest microorganism counts, with a notable growth tendency during both thawing methods. The counter treatment (6H) exhibits higher counts, followed by the fridge treatment (24H). Furthermore, no significant differences were identified between treatments at a significance level of P = 0.09.

No statistically significant differences were found despite the upward and downward behavior in the treatments. On the other hand, the wide separation on the error lines could be explained by the fact that only five replicates were evaluated, increasing the variation between the recollected data. However, this behavior could have happened because some of the chicken tenders evaluated had different conditions; some of them had a greater amount of fat around the muscle, and the size and weight varied as well. These could affect the amount of drip loss and ice melted during the thawing process. Added to these, human error in the amount of water added to the bag could affect the results. It is likely that the microbial load was washed into the water since the sampling involved surface massaging. For this reason, it may be possible that the microbial loads in the thawing of some tenders, as in the case of the Fridge (24H) treatment, could be lower than in the Initial treatment. At the same time, in a study carried out by Marriott et al. (1980), thawing at refrigeration temperatures (5 °C) and at room temperature (25 °C) of ground meat at different times was evaluated. As a result, some major groups of bacteria were not found, such as *Micrococci*, *Pseudomonas*, *Streptococci*, *Staphylococci*, and *Flavobacterium*, among others. However, it was observed that there was a growth of coliforms at room temperature, especially after 8 hours, and it was found that the best way is to thaw at refrigeration temperature for 24 hours. Which can relate with the results obtained shown in Figure 8, where Fridge (24 H) thawing method had lower counts than Counter (6 H).

Figure 8

Enterobacteriaceae growth behavior in relation to blast freezer and freezer storages for the thawing methods



Note. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Initial, Counter (6H), and Fridge (24H) represents the mean of five replicates. Each error line in each column represents the variability in the recollected data.

Psychrotrophs

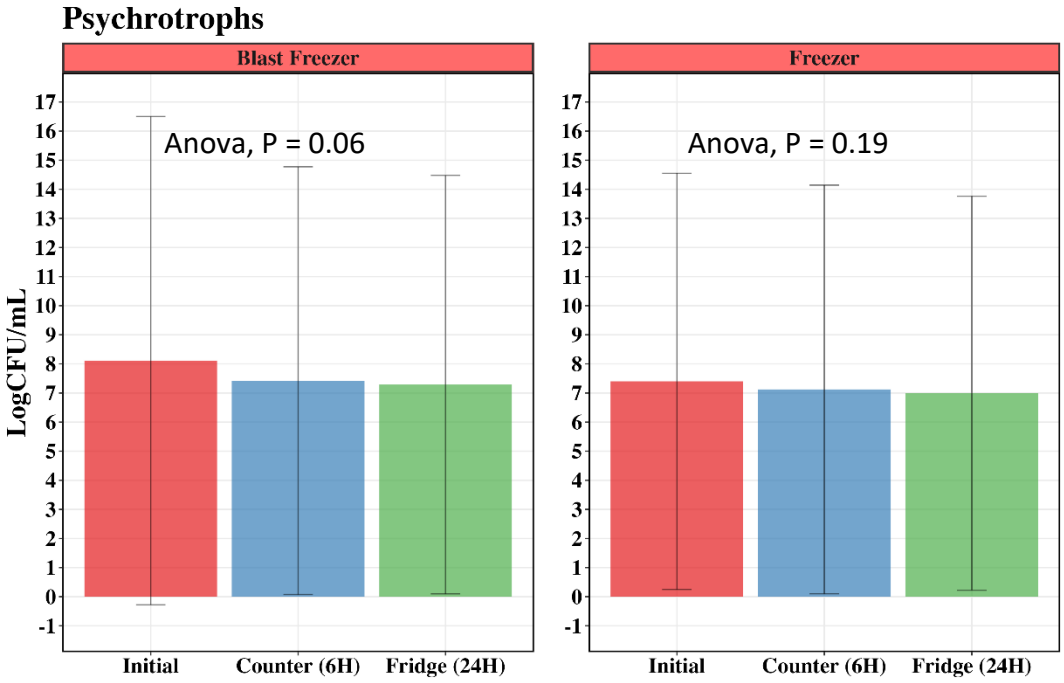
The Figure 9 illustrates the behavior of psychrotrophs in relation to various thawing methods, denoted on the X-axis as Initial, Counter (6 H), Fridge (24 H), and Fridge (24H). The Y-axis represents the logarithmic colony-forming units per milliliter (Log CFU/mL), serving as a measure of psychrotrophic microorganism growth. The figure effectively demonstrates the growth patterns observed after thawing from two distinct storage types (Blast Freezer and Freezer). Furthermore, the figure includes a clear separation of means between the thawing methods and an accompanying error line. In both blast freezer and freezer storage types, a minor decrease in counts was observed across all three treatments. The initial treatment consistently demonstrated the highest count, followed by the counter (6H) treatment, and finally the fridge (24H) treatment. To summarize, no statistically

significant differences were detected between the treatments. The significance level for blast freezer storage was calculated as P = 0.06, whereas for freezer storage, it was determined to be P = 0.19.

The high behavior exhibited by psychrotrophic bacteria can be attributed to their ability to grow at low temperatures, even as low as 0 °C. Examples of bacteria with this capability include *Listeria monocytogenes, Pseudomonas*, and Lactic Acid Bacteria (LAB) (Martin & Fisher, 2000; Schillinger et al., 2006). This phenomenon is further elucidated in a study conducted by Mohammed et al. (2021), that, they assessed various microorganisms present in meat samples, some of which were the aforementioned bacteria, along with *E. coli* O157:H7, *S. aureus*, molds, and yeasts, among others. The samples were stored for defined periods and subsequently thawed overnight at 4 °C. Their findings indicated that freeze-thaw cycles led to increased microorganism counts in all types of meat, including chicken, under thawing conditions.

Figure 9

Psychrotrophs growth behavior in relation to blast freezer and freezer storages for the thawing methods



Note. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Initial, Counter (6H), and Fridge (24H) represents the mean of five replicates. Each error line in each column represents the variability in the recollected data.

Conclusions

The study revealed that psychrotrophic bacteria exhibited an increasing trend across all storage conditions, while Enterobacteriaceae and aerobic mesophilic bacteria displayed a declining pattern.

It was found that during the simulation where the chicken tenders experimented temperature abuse was critical for them to either increase or decrease population, however when stored at a steady temperature the microbial population remained stable, except for Fridge treatment and psychrotrophic bacteria.

It was revealed that only the counts of aerobic mesophilic bacteria differed significantly between treatments, with counter (6H) counts being lower than Initial and fridge (24H) numbers.

It was observed that the counts of Enterobacteriaceae were irregular for Freezer storage, as the counts were affected by a washout of bacteria upon thawing, obtaining lower counts in Fridge (24H) than in frozen chicken tenders (Initial) and a higher load of microorganisms for Counter (6H).

It was concluded that the counts of psychrotrophic bacteria were the highest of the three microorganisms in the two storage temperatures, obtaining the lowest microbiological loads in Fridge (24 H), followed by Counter (6H) and the highest for the frozen chicken tenders.

Recommendations

Increase the number of replicates and thawing methods to have lower variability and higher potential, respectively.

It is recommended for more accurate simulation results to use different storage temperatures that align with consumers' real-life conditions.

Conduct a market study to get a clearer idea of the routine of consumers when buying chicken at the points of sale and thus use more precise times between each of the stages.

Conduct the same study with other types of meat, such as beef and pork, to see if the effect of temperature fluctuations in the cold chain is the same or different.

Carry out the same study, including a sensory analysis to see how temperature fluctuations and different thawing methods affect meat quality, as well as a physicochemical analysis of Aw, product moisture, color, and pH to see how the type of thawing affects its properties.

References

Balcázar, B. J. (2019). *Evaluación del desempeño de métodos microbiológicos normalizados utilizados en el análisis de alimentos para bacterias mesofílicas aerobias y bacterias coliformes totales de acuerdo con la Comisión de Control Analítico y Ampliación de Cobertura en varias matrices* [Tesis de pregrado, Benemerita Universidad Autónoma de Puebla, México]. repositorioinstitucional.buap.mx. http://images.engormix.com/s_articles/pinzon_leche_bacterias.pdf

Baylis, C. L. (2006). 22 - Enterobacteriaceae. In C. d. W. Blackburn (Ed.), *Woodhead publishing in food science, technology and nutrition. Food spoilage microorganisms* (pp. 624–667). CRC; Cambridge : Woodhead Publishing.

Benli, H. (2016). Consumer Attitudes Toward Storing and Thawing Chicken and Effects of the Common Thawing Practices on Some Quality Characteristics of Frozen Chicken. *Asian-Australasian Journal of Animal Sciences*, *29*(1), 100–108. https://doi.org/10.5713/ajas.15.0604

Berry, E. D., & Foegeding, P. M. (1997). Cold Temperature Adaptation and Growth of Microorganisms†. *Journal of Food Protection*, *60*(12), 1583–1594. https://doi.org/10.4315/0362-028X-60.12.1583

Dewey-Mattia, D., Manikonda, K., Hall, A. J., Wise, M. E., & Crowe, S. J. (2018). Surveillance for Foodborne Disease Outbreaks — United States, 2009–2015. *MMWR Surveillance Summaries*, *67*(10), 1. https://doi.org/10.15585/mmwr.ss6710a1

El-Shibiny, A., Connerton, P., & Connerton, I. (2009). Survival at refrigeration and freezing temperatures of Campylobacter coli and Campylobacter jejuni on chicken skin applied as axenic and mixed inoculums. *International Journal of Food Microbiology*, *131*(2), 197–202. https://doi.org/10.1016/j.ijfoodmicro.2009.02.024

Food and Agriculture Organization of the United Nations. (2023). *Chicken meat worldwide production. FAOSTAT*. https://www.fao.org/faostat/en/#data/QCL

Georgsson, F., Þorkelsson, Á. E., Geirsdóttir, M., Reiersen, J., & Stern, N. J. (2006). The influence of freezing and duration of storage on Campylobacter and indicator bacteria in broiler carcasses. *Food Microbiology*, *23*(7), 677–683. https://doi.org/10.1016/j.fm.2005.10.003

Grégory, D., Jean-Claude, R., Catherine, A., & Thierry, S. (2005). *Comparison of TEMPO TC with Conventional Method for the Enumeration of Total Coliforms in Food Samples*. https://www.biomerieux-usa.com/sites/subsidiary_us/files/doc/tempo-tc-vs-iso-1.pdf

Halkman, H.B.D., & Halkman, A. K. (2014). Indicator Organisms. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of food microbiology* (2nd ed., pp. 358–363). Academic Press. https://doi.org/10.1016/B978-0-12-384730-0.00396-7

Ioanna, N. G., Ioannis, K. K., Panagiotis, E. D., & Kyriakos, A. R. (2017). Effect of combination of ozonation and vacuum packaging on shelf life extension of fresh chicken legs during storage under refrigeration. *Journal of Food Engineering*, *213*, 18–26. https://www.sciencedirect.com/science/article/pii/S0260877417302728?ref=pdf_download&fr=RR-2&rr=7c1970b05b8931bf

Janssens, K., Lambrechts, W., van Osch, A., & Semeijn, J. (2019). How Consumer Behavior in Daily Food Provisioning Affects Food Waste at Household Level in The Netherlands. *Foods (Basel, Switzerland)*, *8*(10). https://doi.org/10.3390/foods8100428

Kim, H. C., Baek, K. H., Lee, Y. E., Kang, T., Kim, H. J., Lee, D., & Jo, C. (2022). Using 2D qNMR analysis to distinguish between frozen and frozen/thawed chicken meat and evaluate freshness. *Npj Science of Food*, *6*(1), 1–8. https://doi.org/10.1038/s41538-022-00159-x

Kodaka, H., Mizuochi, S., Teramura, H., & Nirazuka, T. (2005). Comparison of the Compact Dry TC Method with the Standard Pour Plate Method (AOAC Official Method 966.23) for Determining Aerobic Colony Counts in Food Samples: Performance-Tested MethodSM. *Journal of AOAC INTERNATIONAL*, *88*(6), 1702–1713. https://doi.org/10.1093/jaoac/88.6.1702

Li, X., Payne, J.B., Santos, F.B., Levine, J.F., Anderson, K.E., & Sheldon, B.W. (2007). Salmonella Populations and Prevalence in Layer Feces from Commercial High-Rise Houses and Characterization of the Salmonella Isolates by Serotyping, Antibiotic Resistance Analysis, and Pulsed Field Gel Electrophoresis. *Poultry Science*, *86*(3), 591–597. https://www.sciencedirect.com/science/article/pii/S0032579119441187#bib28

Marriott, N. G., Garcia, R. A., Pullen, J. H., & Lee, D. R. (1980). Effect of Thaw Conditions on Ground Beef1. *Journal of Food Protection*, *43*(3), 180–184. https://doi.org/10.4315/0362-028X-43.3.180

Martin, S. E., & Fisher, C. W. (2000). LISTERIA | Listeria monocytogenes. In R. K. Robinson, C. A. Batt, & P. D. Patel (Eds.), *Encyclopedia of food microbiology* (pp. 1228–1238). Academic Press. https://doi.org/10.1006/rwfm.1999.0965

Meng, J., Huang, X., Song, L., Hou, B., Qiao, M., Zhang, P., Zhao, Q., Zhang, B., & Liu, F. (2019). Effect of storage temperature on bacterial diversity in chicken skin. *Journal of Applied Microbiology*, *126*(3), 854–863. https://doi.org/10.1111/jam.14183

Miranda, J. M., Guarddon, M., Vázquez, B. I., Fente, C. A., Barros-Velázquez, J., Cepeda, A., & Franco, C. M. (2008). Antimicrobial resistance in Enterobacteriaceae strains isolated from organic chicken, conventional chicken and conventional turkey meat: A comparative survey. *Food Control*, *19*(4), 412–416. https://doi.org/10.1016/j.foodcont.2007.05.002

Mohammed, H. H. H., He, L., Nawaz, A., Jin, G., Huang, X., Ma, M., Abdegadir, W. S., Elgasim, E. A., & Khalifa, I. (2021). Effect of frozen and refrozen storage of beef and chicken meats on inoculated microorganisms and meat quality. *Meat Science*, *175*, 108453. https://doi.org/10.1016/j.meatsci.2021.108453

Nazarowec-White, M., & Farber, J. M. (1997). Incidence, Survival, and Growth of Enterobacter sakazakii in Infant Formula. *Journal of Food Protection*, *60*(3), 226–230. https://doi.org/10.4315/0362-028X-60.3.226

Olgunoglu, I. A. (2010). Effects of Blast-Freezing and Glazing on Microbiological Changes of Skinless and Skinned Tench (Tinca tinca L. 1758) Fillets During Frozen Storage (-18 degrees C). *Pakistan Journal of Zoology*, *42*(4), 365–370. https://www.researchgate.net/publication/228506133_Effects_of_Blast-Freezing_and_Glazing_on_Microbiological_Changes_of_Skinless_and_Skinned_Tench_Tinca_tinca_L_1758_Fillets_During_Frozen_Storage_-18_degrees_C

Paseto Fernandes, R. d. P., Alvarenga Freire, M. T., Maluf de Paula, E. S., Sayuri Kanashiro, A. L., Pinto Catunda, F. A., Fernandes Rosa, A., de Carvalho Balieiro, J. C., & Trindade, M. A. (2014). Stability of lamb loin stored under refrigeration and packed in different modified atmosphere packaging systems. *Meat Science*, *96*(1), 554–561. https://doi.org/10.1016/j.meatsci.2013.08.005

Pensiri, K., Luigi, P., Jorge, C., Susanne, K., Saowakon, W., & Anders, H. K. (2019). *Changes in the Quality of Chicken Breast Meat due to Superchilling and Temperature Fluctuations during Storage*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7005396/

Pérez Álvarez, S., Coto Arbelo, O., Echemendia Pérez, M., & Ávila Quezada, G. (2015). Pseudomonas fluorescens Migula, ¿control biológico o patógeno? *Protección Vegetal*, *30*(3), 225–234. http://scielo.sld.cu/pdf/rpv/v30n3/rpv08315.pdf

Pinzon Fernandez, A. (2006). *Determinación del índice de Bacterias mesófilas Aerobias Presentes en la Leche Cruda Versus Leche Pasteurizada que se Comercializan en la Zona Urbana de la Ciudad de Popayan* [Tesis de posgrado]. Universidad Nacional Abierta y a Distancia, Popayán. http://images.engormix.com/s_articles/pinzon_leche_bacterias.pdf

Rajiv Kumar, Dimpi Dave, Swagatika Mishra, & Rajesh R Nair (2020). A comparative study of automated TEMPOÂ® rapid method with IS/ISO method for enumeration of microorganisms in different dairy products. *Indian Journal of Dairy Science*, *73*(3). https://epubs.icar.org.in/ejournal/index.php/IJDS/article/view/102277

Rogers, L., Power, K., Gaora, P.Ó., & Fanning, S. (2016). Escherichia coli and Other Enterobacteriaceae: Occurrence and Detection. In B. Caballero (Ed.), *Encyclopedia of food and health* (pp. 545–551). Academic Press. https://doi.org/10.1016/B978-0-12-384947-2.00259-2

Russel, S. M. (1995). The Effect of Refrigerated and Frozen Storage on Populations of Mesophilic and Coliform Bacteria on Fresh Broiler Chicken Carcasses. *Poultry Science*, *74*(12), 2057–2060. https://doi.org/10.3382/ps.0742057

Russell, N. J. (2002). Bacterial membranes: the effects of chill storage and food processing. An overview. *International Journal of Food Microbiology*, *79*(1), 27–34. https://doi.org/10.1016/S0168-1605(02)00176-9

Schillinger, U., Holzapfel, W. H., & Björkroth, K. J. (2006). 20 - Lactic acid bacteria. In C. d. W. Blackburn (Ed.), *Woodhead publishing in food science, technology and nutrition. Food spoilage microorganisms* (pp. 541–578). CRC; Cambridge : Woodhead Publishing. https://doi.org/10.1533/9781845691417.5.541

Suhren, G. (2020). Producer Microorganisms. In R. C. McKellar (Ed.), *Enzymes of psychrotrophs in raw food* (1st ed., pp. 3–34). CRC Press. https://doi.org/10.1201/9781003068457-2

Thomas, S. B. (1969). Methods of assessing the psychrotrophic bacterial content of milk. *The Journal of Applied Bacteriology*, *32*(3), 269–296. https://doi.org/10.1111/j.1365-2672.1969.tb00974.x

U.S Food and Drug Administration. (2018). *Refrigerator and freezer storage chart*. https://www.fda.gov/media/74435/download

United States Department of Agriculture. (2019). *Chicken from Farm to Table | Food Safety and Inspection Service*. https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/poultry/chicken-farm-table

Walter de Santana, E. H., Lima Luiz, L., Pasquim, P. d. S., Bertachi Pinto, L. d. F., Bergonse Pereira, F. d. A., Freitas Barone Gasparini, G. B., Lorenzetti, E., Bruzaroski, S. R., & Ito Eleodoro, J. (2020). Psychrotrophic microorganisms in raw milk and the cheese quality. *Research, Society and Development*, *9*(9), e127997217-e127997217. https://doi.org/10.33448/rsd-v9i9.7217

Appendices

Appendix A

Aerobic Mesophilic Bacteria growth behavior in relation to blast freezer and freezer storages for the thawing methods

	Blast Freezer	Freezer
Treatments	Log CFU/mL ± SD	Log CFU/mL ± SD
Initial	3.272 ± 3.25[a]	3.174 ± 2.79[a]
Counter (6H)	1.888 ± 1.18[b]	1.974 ± 1.46[c]
Fridge (24 H)	2.668 ± 3.11[ab]	2.444 ± 2.35[b]
P	<0.05	<0.05
CV%	94.28	87.16

Note. S.D = Standard Deviation. CV% = Coefficient of Variation. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Initial, Counter (6H), and Fridge (24H) represents the mean of five replicates. [a-c]: Different letters in each column indicate significant differences ($P < 0.05$) between treatments.

Appendix B

Enterobacteriaceae growth behavior in relation to blast freezer and freezer storages for the thawing methods

	Blast Freezer	Freezer
Treatments	Log CFU/mL ± S.D.	Log CFU/mL ± S.D.
Initial	0.105 ± 0.25	0.096 ± 0.30
Counter (6H)	1.690 ± 1.83	2.007 ± 2.16
Fridge (24 H)	0	0.904 ± 0.66
P	<0.05	<0.05
CV%	87.67	73.50

Note. S.D = Standard Deviation. CV% = Coefficient of Variation. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Initial, Counter (6H), and Fridge (24H) represents the mean of five replicates.

Appendix C

Psychrotrophs growth behavior in relation to blast freezer and freezer storages for the thawing methods

	Blast Freezer	Freezer
Treatments	Log CFU/mL ± SD	Log CFU/mL ± SD
Initial	7.758 ± 7.24	7.608 ± 7.15
Counter (6H)	7.357 ± 7.18	7.194 ± 7.02
Fridge (24 H)	7.604 ± 7.30	7.036 ± 6.76
P	0.06	0.19
CV%	95.67	95.90

Note. S.D = Standard Deviation. CV% = Coefficient of Variation. Log CFU/mL: logarithms of concentration of Colony – Forming Units per milliliter in a sample. Each microbiological count of Initial, Counter (6H), and Fridge (24H) represents the mean of five replicates.