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Influence of Organic Matter from Native Fish on the Antimicrobial Efficacy of Sodium Hypochlorite (NaClO) in Reducing *Salmonella* spp. Population

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Abstract: *Salmonella* spp. is not part of the fish microbiota, being introduced through contaminated water or improper handling. In slaughterhouses, five parts per million (ppm) of free chlorine is recommended in the washing step to eliminate this pathogen. However, *Salmonella* spp. is still detected in fish processing plants due to persistent contamination as a result of chlorine–organic matter interactions that reduce chlorine effectiveness. Therefore, this study aimed to test whether organic matter contained in culture media and fish washes interferes with the efficacy of chlorine at 2 and 5 ppm, and what is the time of action required to inactivate ATCC and wild strains of *S. typhimurium*. For this, the elimination or survival of these strains was investigated when exposed to chlorinated solution for different durations (0, 5, 30, 60, 90 and 120 min) in culture medium, 0.85% saline solution and fish wash (in natura and sterilized). The results showed that the use of 5 ppm of free chlorine is efficient, even when the chlorine remains in contact with the organic matter (washed from the fish), as it reduced the bacterial population by ~5 log log10 CFU/mL of *Salmonella* spp., showing that this reduction was due to interactions, as long as the chlorine action time was 30 min.

Keywords: *Salmonella typhimurium*; fish; sodium hypochlorite; exposure time; organic matter; bacterial reduction

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1. Introduction

Aquaculture is one of the food production sectors that has grown the most in recent years [1]. This sector was responsible for the production of around 86.5 million tons in 2018 [2]. A total of 221 countries have some commercial activity involving fish and fishery products, representing 78% of international trade [3]. Brazil ranks eighth in the world; however, it is among the top producers of freshwater fish, with a growing trend due to Brazil being the country with the largest amount of freshwater available [4]. Given the availability of water resources and the favorable climate in some countries for fish production, this product becomes a promising alternative source of protein for a large portion of the world's population [5–7].

Fish and fish products represent an important source of consumption for the population, in addition to being nutritionally rich, with a high content of polyunsaturated fats and proteins of high biological value and easy digestibility [8]. However, due to their

intrinsic characteristics, such as high water activity, pH and nutrient availability, fish have a higher spoilage rate compared to other animal products, which is often a limiting factor in consumer purchases [9]. Therefore, controlling the initial microbial load, ensuring the quality of the raw material and maintaining adequate processing and storage conditions are crucial factors in guaranteeing a product with a longer shelf life and consequently a lower risk of contamination for the consumer [10].

In this context, several pathogens may be present in fish, including *Salmonella* [11]. An important point to note is that *Salmonella* spp. is not a biological contaminant that is part of the natural microbiota of fish [12]. However, contamination by *Salmonella* spp. in fishery products can stem from environmental contamination where the fish are grown or harvested [13], or due to failures in handling during harvesting or processing [14,15].

Among the different serotypes of *Salmonella*, the *Typhimurium* serotype is present in various animal-origin matrices, being the most prevalent and having a cosmopolitan distribution [16]. This serotype is considered the main cause of human salmonellosis cases in North America and Oceania, regardless of the animal source [16,17]. Additionally, it is the second most common *Salmonella* serotype in Europe [18]. An epidemiological study of *Salmonella typhimurium* in Brazil, covering clinical samples and foods (poultry, chicken, pork and beef), was conducted in the regions of São Paulo, Santa Catarina, Paraná, Mato Grosso do Sul, Rio Grande do Sul, Goiás and Bahia [15,19–21]. However, studies on fish are reported only in the states of Mato Grosso and Maranhão [15,20,21]. In the study by [15], the *Typhimurium* serotype was detected in samples collected at a fish slaughterhouse during the transportation, washing with chlorinated water, evisceration, skinning and filleting stages. Therefore, control measures must be developed, integrated into the process and monitored in the processing environment, primarily to control foodborne pathogens.

As a form of microbiological control, some regulations establish minimum requirements for quality and identity standards for fish [22,23]. In the animal product industry, chlorine and its derivatives are widely used agents to eliminate pathogenic microorganisms [24]. According to the Ministry of Agriculture, Livestock and Supply of Brazil [25], the maximum recommended level is up to five parts per million (ppm) of free residual chlorine. Washing carcasses with sodium hypochlorite is the main step in controlling the presence of pathogens in fish. Hypochlorous acid, a substance present in chlorine, is an effective oxidant with a neutral charge capable of easily penetrating the external membrane of bacteria, which has a negative charge, disrupting metabolic processes and breaking the cell wall [26–29].

Even with permission to use up to 5 ppm of free residual chlorine in fish slaughterhouses, some studies conducted in the state of Mato Grosso (the second-largest producer of native fish in Brazil [30]) have shown that using chlorine at this concentration may not be effective in controlling *Salmonella* spp. in fish. This hypothesis is based on the results found by Santos et al. (2018) [31], who detected the presence of *Salmonella* in culture water and fish. Furthermore, the presence of *Salmonella* was confirmed at all stages of the processing environment [15] and in fish exposed for commercialization [32].

Studies focused on controlling the presence and growth of pathogenic *Enterobacteriaceae* species and serotypes using chlorine and its derivatives in animal products are documented. Research involving eggs and panga fish (*Pangasius hypophthalmus*) using chlorine in the form of chlorine dioxide and hypochlorous acid to combat *Salmonella enteritidis* (ATCC) and wild *Escherichia coli* in Republic of Korea and Vietnam has been reported [27,33]. However, in our review, we observed no studies utilizing sodium hypochlorite against *Salmonella* spp. in fish.

Therefore, the objective of the present study was to evaluate the population reduction rate of *S. typhimurium* and the antimicrobial action of NaClO at concentrations of up to 5 ppm of free chlorine, both in the presence and absence of organic matter from native fish and culture medium.

2. Materials and Methods

2.1. Strains of S. typhimurium

In this study, *S. typhimurium* (ATCC 10749) was kindly provided by the National Reference Laboratory for the Diagnosis of Enteric Bacteria, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (FIOCRUZ). A wild strain of *S. typhimurium* (P06), isolated from fish captured in the state of Mato Grosso [15] of Tambaqui (*Colossama macropomum*) was also used. The *Typhimurium* serotype was chosen because it is considered one of the main serotypes causing infections in humans globally [34]. The strains were preserved in brain heart infusion broth (BHI; KASVI®, Madrid, Spain) containing 20% (*v*/*m*) glycerol (Labsynth®, São Paulo, Brazil) and were stored at −86 °C. During the analysis period, the strains were reactivated in 20 mL of brain heart infusion broth (BHI; KASVI®, Madrid, Spain) and incubated at 37 °C for 24 h.

2.1.1. Preparation of Chlorinated Water and Monitoring of Total and Free Chlorine

For the inactivation tests using NaClO, a commercial solution at an initial concentration of 12% (Chloro, MT®, Cuiabá, Brazil) was used. Concentrations of 2 and 5 ppm were achieved by diluting the concentrated solution in ultrapure water (we add 70 µL of the concentrated solution to 300 mL of water for the first dilution, and we add 30 µL of the concentrated solution to 300 mL of water for the second dilution), in an environment at a temperature of approximately 27 °C. To ensure that the concentrations of total chlorine (CT) and free chlorine (CL) were as desired, the concentrations were measured using a multiparameter chlorine meter Kit Micro 7+/Micro20. (ASKO®, Lidköping, Sweden). The entire procedure was performed according to the manufacturer's recommendations, and the results were expressed in parts per million.

2.1.2. Experimental Design

The survival of *S. typhimurium* strain ATCC 10749 and strain P06, isolated from fish, was evaluated based on chlorine concentration in parts per million (ppm), specifically, 2 and 5 ppm at different exposure times (0, 5, 30, 60, 90 and 120 min). We also evaluated three modes of exposure: the strain contained in brain heart broth, strain contained in saline solution and strain contained in saline solution plus in natura fish washing and autoclaved fish washing (as shown in Figure 1A,B). For each treatment, a negative control was conducted, involving serial dilutions of the strain and subsequent dilutions and plating at concentrations ranging from 10^4 to 10^8 CFU/mL. The count obtained during plating was an average population of 10^8 CFU/mL. The purity of these strains was verified through inoculation on selective media such as Brilliant Green Agar and Xylose Lysine Deoxycholate Agar.

2.1.3. Susceptibility of *S. typhimurium* to 5 ppm and 2 ppm of Free Chlorine

To assess the susceptibility of the *S. typhimurium* strains (ATCC 10749 and P06), 1 mL of the suspension of these strains in culture medium (Figure 1A) or in saline solution (Figure 1B) was added to tubes containing 9 mL of chlorinated water at 2 and 5 parts per million (ppm). This mixture of chlorinated water and microorganism was maintained at room temperature (~27 °C) for various durations: 0 (T0), 5 (T5), 30 (T30), 60 (T60), 90 (T90) and 120 (T120) min. After each treatment, the antimicrobial activity of NaClO was neutralized by adding 0.1% (*v*/*v*) sodium thiosulfate (Labsynth®, São Paulo, Brazil) to the tube. To investigate the action of chlorine on the strains, dilution of the solution (chlorinated water plus microorganism) and decimal fractions from 10^1 to 10^4 were prepared, including 100 µL of the solution in 900 µL of saline solution (chlorinated water plus microorganism). Then, 0.1 mL of each dilution was spread onto nutrient agar plates (KASVI, Madrid, Brazil) and incubated at 37 °C for 24 h, after which the colonies were counted.

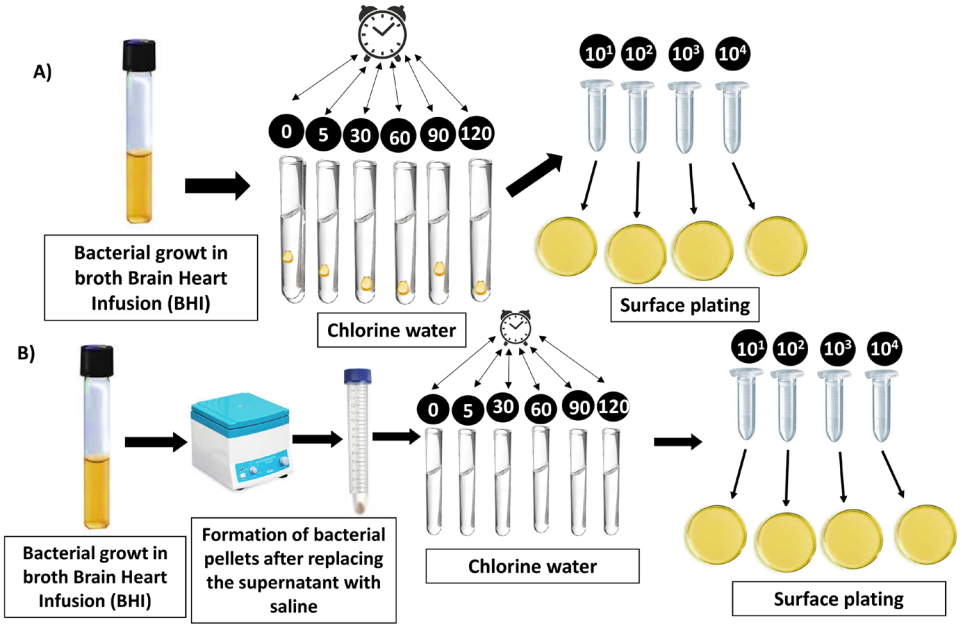

Figure 1. Exposure of *S. typhimurium* strains (ATCC and wild P06) to 2 and 5 ppm of free chlorine at 0, 5, 30, 60, 90 and 120 min in different treatments: (**A**) exposure of strains in culture medium (BHI); (**B**) exposure of resuspended strains in saline.

2.1.4. Effect of Organic Matter on the Presence of Free Chlorine and Its Bactericidal Effect on *S. typhimurium* (Strain ATCC 10749 and Wild-Type P06)

The presence of organic matter was simulated using surface washing from a Tambaqui fish (*Colossama macropomum*) that had already been slaughtered. This involved surface washing of the fish at a 1:4 (v/w) ratio, using 400 mL of saline solution and including a sterile polystyrene bag with a fish weighing approximately 1600 g [35], as adapted. The amount of washing was standardized to concentrations of 5 and 10% in the final solution containing bacteria and culture medium and/or saline solution. The amount of chlorine that did not interact with the organic matter was measured using the Micro 7 kit (Akso, RS-BR), quantifying total chlorine (CT) and free chlorine (CL). The readings were performed in triplicate and expressed in ppm. To define the percentage of washing to be used in the experiment, a linear regression was conducted to identify the decline in the action of 5 ppm of chlorine in contact with different percentages of washing (1 to 10%). After this step, it was decided to use 5% washing during the evaluation of chlorine exposure time, mimicking fish production and the microbial load naturally present in this process. This percentage of organic matter has been used in previous studies [26].

Subsequently, the action of chlorine on the strains resuspended in saline solution was investigated (Figure 1B). Here, the culture medium (BHI) was removed by centrifugation at 450× *g* for 2 min, and the pellet was resuspended in saline solution at a concentration of 0.85% in the same volume as the culture medium (10 mL). To this, 0.45 mL (5%) of both in natura fish wash and fish wash (autoclaved) was added. The mixture was then exposed to 8.55 mL of chlorinated water solution at 2 and 5 (±0.5) ppm of free chlorine. The results were determined by counting the survival of the *S. typhimurium* strains in colony counts after exposure to the treatments, followed by inoculation in the nutrient agar at 37 °C for 24 h. After this period, the colonies were counted, and the final population was expressed in Log_{10} CFU/mL (colony-forming unit).

2.2. Statistical Treatment

The data were analyzed using Student's *t* test to compare whether there was a statistically significant difference between the two culture media (BHI culture medium and saline solution). In addition, the results of the bacteria's exposure times to chlorine were

analyzed using ANOVA to determine whether there was a significant difference between all treatments performed. Following the ANOVA, the post hoc Fisher's least significant difference (LSD) test was conducted with 95% confidence, comparing the treatments performed and the exposure times of the strains to the 5 ppm chlorinated solution. Finally, a linear regression of free and total chlorine as a function of the percentage of organic matter (fish wash) present in the medium and the decrease in free chlorine present in the solution was also performed. The equation, along with the adjusted R^2 values, was used to assess the quality of the results.

3. Results

We found that the use of saline solution favored greater bacterial death and that the use of culture medium resulted in an inactivation plateau, with 2 and 5 ppm reaching a similar inactivation rate (approximately 2 Log for 2 ppm and 2.5 Log for 5 ppm, from an initial average population of around 7 Log) for the P06 wild strain, showing no statistically significant differences between exposure times. The *S. typhimurium* strain (ATCC 10749) also showed little reduction (around 1.5 Log for both 2 ppm and 5 ppm from an initial population average of around 7 Log) that was not significantly different between exposure times. This could be because the reductions were minimal due to the culture medium (BHI), which acted as a chlorine scavenger, as shown in Figure 2.

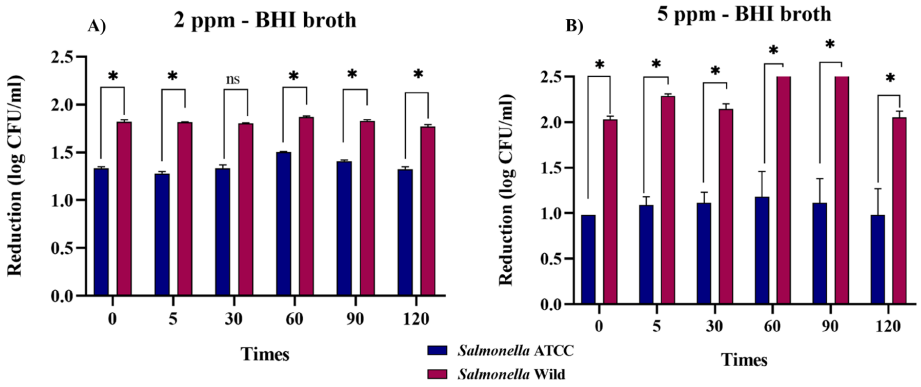

Figure 2. Inactivation (Log_{10} CFU/mL) of *Salmonella typhimurium* strains (ATCC 10749 and the P06 wild strain) when exposed to 2 ppm (**A**) and 5 ppm (**B**) of free chlorine in the time periods (T0 to T120 min) when included in culture medium. Student's *t* test; * indicates case comparison differences between treatments; ns indicates that there was no difference in time periods (T0 to T120).

When we used the saline solution, we found that the difference between 2 and 5 ppm was considerably significant, confirming the effect of organic matter. When the strain of *S. typhimurium* (ATCC 10749) and the wild strain P06 were exposed to a 2 ppm chlorinated solution, the results showed different behaviors, with a statistically significant difference. The *S. typhimurium* wild P06 strain, surviving in the production environment, showed greater survival and adaptation to the effects of chlorine. Furthermore, a greater reduction from time zero (T0) was revealed with the *S. typhimurium* strain (ATCC 10749) (Figure 3A).

Regarding the treatment with 5 ppm, a reversal in the reduction was observed, with the *S. typhimurium* strain (ATCC:10749) being more sensitive compared to the *S. typhimurium* wild P06 strain. The ATCC 10749 strain showed a reduction of 6.17 Log_{10} CFU/mL from 30 min (T30) onward, while the wild-type strain showed a reduction greater than 4 logs (4.42 Log_{10} CFU/mL) for the same duration, eventually reaching a reduction of up to 5 Log_{10} CFU/mL at 120 min (T120). However, according to the statistical analysis performed, the treatment of the strain in the saline solution at various times (T5, T30, T60, T90 and T120) at 5 ppm did not differ statistically and was still considered the most effective treatment ($p \leq 0.5$).

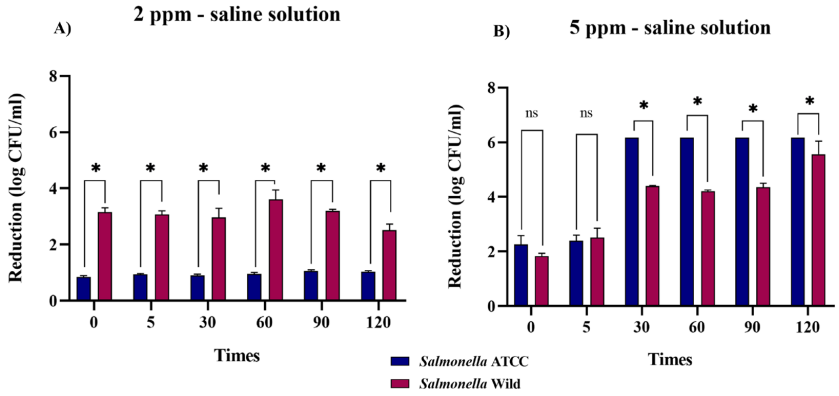

Figure 3. Inactivation (Log_{10} CFU/mL) of *Salmonella typhimurium* (ATCC 10749 and wild P06) when exposed to 2 ppm (**A**) and 5 ppm (**B**) of free chlorine in the time periods (T0 to T120 min) when included in culture medium or saline solution. Student's *t* test; * indicates case comparison differences between treatments; ns indicates that there was no difference in time periods (T0 to T120 min).

When comparing the results of the population reduction (Log_{10} CFU/mL) in *S. typhimurium* (ATCC 10749) and *S. typhimurium* P06 upon exposure to a 5 ppm chlorinated solution from time zero (T0) to time 120 min (T120) and between the strains contained in washing of sterilized and non-sterilized fish (organic matter) (Figure 4A,B), we can observe a very distinct reduction behavior related to the sterile and non-sterile organic matter content. Additionally, the behavior of population reduction in the *S. typhimurium* strains (ATCC 10749) and P06 was noticeably different, as seen in Figure 4.

It is noted that the population reduction was greater in the P06 wild strain of *S. typhimurium*, with reductions of approximately 5 Log_{10} CFU/mL in non-sterile organic matter, showing a statistically significant difference and greater reduction compared to the strain (ATCC 10749). The strain contained in sterile organic matter showed a total reduction from 5 min (T5) onwards but did not differ statistically from time 5 min (T5) to 120 min (T120), as observed in Figure 4A. This was considered a statistically more effective treatment compared to the strain (ATCC 10749).

In addition, we also performed a regression analysis and found that free chlorine decreased linearly in accordance with the inclusion of fish washing. This demonstrates that the profile of chlorine reduction in relation to the percentage of organic matter was linear ($R^2 = 0.96$). In other words, the greater the percentage of organic matter (fish washings) in contact with chlorine, the greater the decline in chlorine availability. For instance, 2% fish washings in contact with chlorinated water at 5 ppm reduced its availability to 3.94 ppm. Similarly, chlorine in contact with 5% washing reduced the availability of residual chlorine to 1.43 ppm, impacting the efficiency of this sanitizer, as seen in Figure 5.

Finally, when comparing all of the treatments and the times of exposure of the strains to the 5 ppm chlorinated solution, the wild strain P06, which was contained in the autoclaved wash from time T5, was shown to be the most effective treatment according to the mean test performed. Additionally, the treatment from times T5 to T120 did not present a statistically significant difference, as shown in Figure 6.

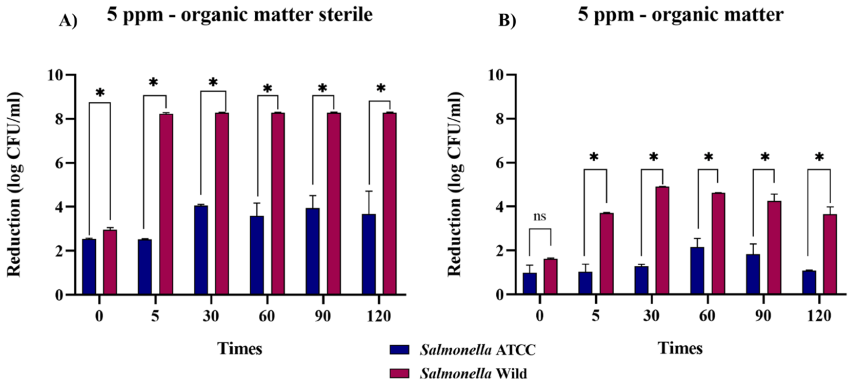

Figure 4. Inactivation (Log_{10} CFU/mL) of *Salmonella typhimurium* (ATCC 10749 and wild P06) when exposed to 5 ppm of free chlorine from the inoculum in saline solution with the addition of 5% sterilized fish wash (**A**) and non-sterilized fish wash (**B**). * Indicates case comparison differences between treatments; ns indicates that there was no difference in time periods (T0 to T120 min).

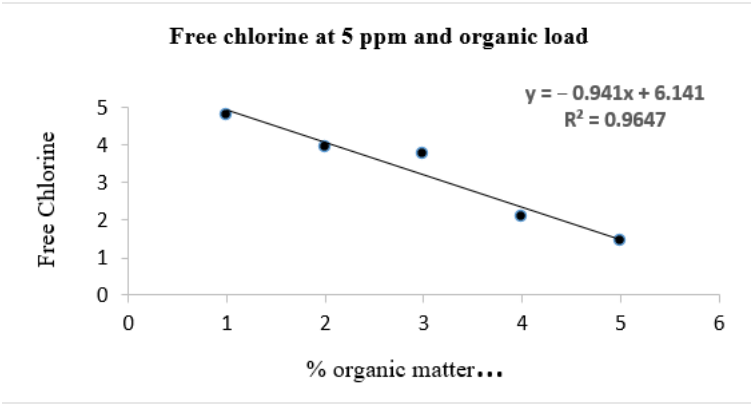

Figure 5. Decline of chlorine action at 5 ppm in contact with different percentages of fish washing.

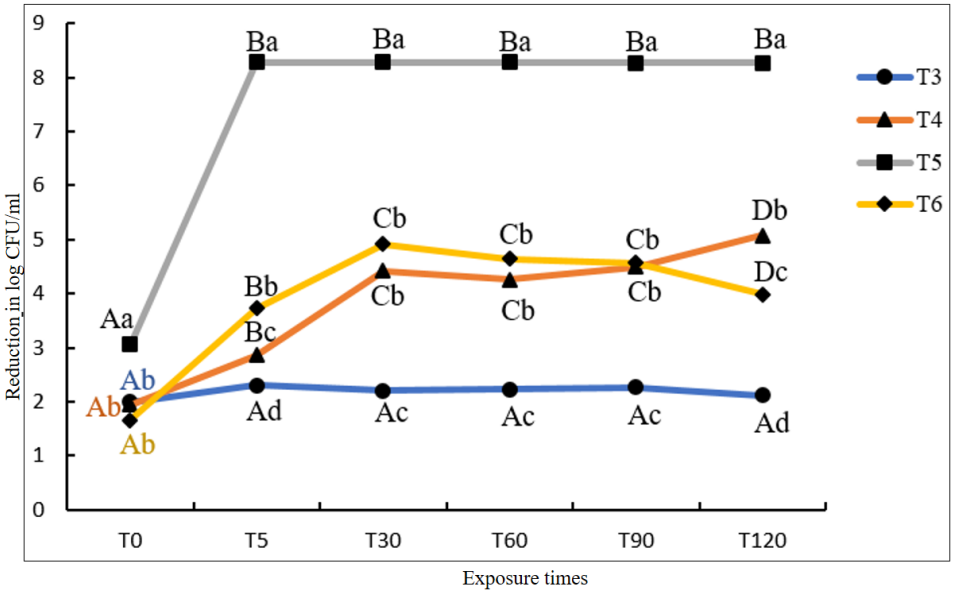

Figure 6. Comparison of the inactivation of the *Salmonella typhimurium* P06 strain due to the treatments (T3 = culture medium; T4 = saline solution; T5 = autoclaved washing; T6 = washing without autoclaving) in relation to the exposure time that varied from T0 to T120 min. Equal uppercase and lowercase letters indicate treatments that did not differ from each other.

4. Discussion

Several studies have already shown contamination by *Salmonella* in fish slaughterhouses, including in the raw material, during processing stages, and in the final product exposed for commercialization [15,30,31]. This highlights a problem that deserves further investigation, especially since in Brazil, chlorinated products are the only sanitizing agents permitted by legislation for washing fish during industrial processing [25].

Free chlorine at concentrations of 2 and 5 ppm led to a reduction in the bacterial load of *Salmonella typhimurium* strains from 5 to 120 min (Figures 3 and 4). However, it was observed that this reduction varied depending on the presence of organic matter. In the absence of culture medium (peptone and infusion of brain heart (BHI) broth), the antimicrobial action of NaClO was more effective, achieving a greater bacterial reduction of approximately 4 Log_{10} CFU/mL for *Salmonella typhimurium* ATCC and 6 Log_{10} CFU/mL for the strain P06, particularly at a concentration of 5 ppm (Figure 4B).

The greater survival of *Salmonella* in the treatment using BHI is due to the presence of organic matter in the culture medium (BHI), which directly interacts with chlorine. This effect can be attributed to the inactivation of some chlorine molecules by organic matter, as chlorine is a strong oxidant that reacts quickly with organic substances [33], causing a rapid decline in free chlorine concentration and consequently a decrease in bactericidal efficiency [27,36–40]. This is a major concern for public health. According to Venkitanarayanan et al. (2002) [41], the use of a disinfectant chemical agent such as chlorine should be capable of reducing at least 5 Log_{10} CFU/mL for pathogenic bacteria like *Salmonella*.

The stage of receiving fish at slaughterhouses often becomes one of the most critical, as it is at this stage that the fish arrive with undesirable organic substances such as soil, mud, dust, grease and other dirt [3]. These substances can interact directly with chlorine, potentially rendering the washing step with chlorinated water ineffective in combating *Salmonella*. According to Gomes et al. (2020) [38], organic matter can directly interfere by reducing the effectiveness of chlorine.

To verify the interaction of chlorine with the organic matter from fish, we carried out experiments using both fresh (in natura organic matter) and autoclaved fish washing (Figure 4A,B). This experiment revealed that reductions in bacterial population (*S. typhimurium* ATCC 10749 and wild P06) were smaller when using fresh fish washing compared to autoclaved fish washing. Given that chlorine, primarily in the form of hypochlorous acid (HOCI), interacts with bacterial proteins by generating oxidative reactions of HOCI with the amino acids such as tryptophan, tyrosine, histidine and sulfur-containing amino acids like cysteine and methionine, as well as peptides and protein residues [42], we presume that a similar interaction could have occurred between chlorine and the proteins in the fresh fish wash. This would immobilize some HOCI molecules, a phenomenon not observed when using the sterilized wash (Figure 3A). The heat treatment in the autoclaving process might prevent protein–protein biding [43], thereby leaving more chlorine free to react with the amino acids and proteins of the bacteria. This justifies the greater reduction in the strains contained in the sterilized wash (Figure 3A) [44,45].

Our study aimed to verify the ability of free chlorine to reduce the population of *Salmonella typhimurium* in fresh fish wash. We observed little reduction, particularly in the ATCC strain. In this regard, we performed a regression analysis and found that the greater the percentage of organic matter (fish wash) in contact with chlorine, the greater the drop in chlorine availability, decreasing from 5 ppm to 1.43 ppm of free chlorine (Figure 6). This decreased occurred linearly as the concentration of fish wash varied from 1% to 5% organic matter. A similar reduction was verified in a study involving chicken organic matter, where the presence of 5% organic matter reduced the availability of free chlorine approximately ten times [36].

Considering that organic matter reduces the effectiveness of free chlorine and its antimicrobial action, it is crucial for fish slaughterhouses to adopt new strategies throughout the fish processing stages to effectively control *Salmonella* spp. Our results highlight the

need to reduce organic matter from fish to enhance the antimicrobial action of free NaClO. This could be achieved by implementing an initial prewash on the surface of the fish to remove environmental dirt from harvesting and transport. This prewash should be carried out before the washing with hyperchlorinated water with 5 ppm free NaCl, which should be maintained for a minimum of 30 min to ensure the maximum reduction in *Salmonella*.

Observing the behavior of the different treatments used in this study (Figure 6), it was found that treatment T5 (autoclaved washing) and T4 (inoculum in saline solution) achieved a reduction above 5 Log_{10} CFU/mL (5.08) at 120 min, with treatment T5 even reaching 100% reduction (Figure 6). However, these treatments may not reflect the reality of a slaughterhouse setting. On the other hand, treatments T6 (washing in natura) and T4 (saline solution) are more representative of a slaughterhouse environment, showing a reduction of approximately 5 Log_{10} CFU/mL at 30 min of exposure for both treatments (p 0.005). Furthermore, no statistically significant difference was observed between these treatments. The T4 treatment could also closely mimic real-world conditions, as it represents a type of precleaning that could be applied to a fish to remove dirt such as mud, earth, fat and other organic compounds.

Comparing the strains of *Salmonella typhimurium,* P06 and ATCC (10749), the P06 strain exhibited a higher survival rate against chlorine in the 5 ppm treatment in saline solution, with a reduction below 5 Log_{10} CFU/mL at 30 min of exposure. This is in contrast to the ATCC strain of *S. typhimurium* (ATCC 10749), which showed a reduction above 6 Log_{10} CFU/mL for the same duration (Figure 2). Given that the *Salmonella typhimurium* P06 used in this study originated from a fish slaughterhouse where it was previously exposed to 5 ppm of chlorine, the high survival and adaptive capacity of the strains when subjected to such treatments could explain this resistance [46].

This adaptive capacity of enteric pathogens, including *Salmonella enterica*, is manifested under stress conditions, such as osmotic shock, which can induce the expression of the *RpoS* gene [47]. The *RpoS* gene is considered one of the most important alternative sigma factors necessary for the maximum survival of enteric pathogens under stress conditions, including *Salmonella enterica* [48]. Previous studies have focused on the *RpoS* gene and its role in survival under various stress conditions to which *Salmonella typhimurium* strains are exposed [49]. The strain of *Salmonella typhimurium* studied here was isolated from fish after washing with chlorine, having been previously exposed to chlorine stress [15]. Therefore, the survival of the *S. typhimurium* P06 strain, as studied by [15], may be mediated through the presence of some type of resistance gene. Notably, *E. coli* strains carrying the *RpoS* gene, with prior contact with chlorine, have been observed to express resistance to this sanitizer [50,51].

5. Conclusions

Considering the processing routine in fish slaughterhouses, it is clear that the fish reception stage is considered critical, as the fish arrives with a high load of organic matter (such as soil, earth and mud). This load can influence the subsequent washing step with hyperchlorinated water at 5 ppm, which normally occurs in a short period. All of these factors contribute to reducing the bactericidal action of chlorine against *Salmonella*. Therefore, it was possible to observe that 5 ppm of free chlorine, even in contact with organic matter, was effective in reducing *Salmonella*, as long as the chlorine had an action time of 30 min. For best results, it is suggested that it would be necessary to previously wash the surface of the fish to remove organic matter, and only then proceed with washing with hyperchlorinated water at 5 ppm.

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