

Growth Prediction of *Aeromonas hydrophila* in Fresh Cheese Stored at 4°C

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Abstract

The aim of this study was to determine the behavior of *Aeromonas hydrophila* in “Minas Frescal” cheese during storage at 4°C, 8°C and 12°C using a regression model and to predict the development of this microorganism during 24 days of storage. Cheese was vacuum-packed and then stored at the study temperatures. *A. hydrophila* and aerobic heterotrophic psychrotrophic bacteria (AHPB) were quantified during storage, water activity (wa) and pH were analyzed. Then, the microbiological counts were submitted to regression analysis and the averages were analyzed using Pearson's correlation with the wa and pH parameters. Results showed that *A. hydrophila* developed significantly at refrigeration temperatures, with high growth rates at 4°C and 8°C, in contrast to those observed at 12°C. There was a slight variation in the wa and pH results and AHPB presented good performance at all the temperatures analyzed. The models obtained fitted better at a temperature of 4°C and were able to predict the growth of *A. hydrophila* with a model fit of 90% ($p < 0.001$). In conclusion, the model obtained for predicting the growth of *A. hydrophila* at 4°C was accurate up to 13 days after the product was manufactured, showing that temperature control was crucial for maintaining product quality; wa and pH were not parameters for quality control.

Keywords: food safety, emerging pathogens, dairy products, microbial competition

1. Introduction

Aeromonas hydrophila is gram negative and grows in a wide range of environmental conditions, such as temperatures from 1°C to 40°C, pH between 4 and 10 and salt concentrations of up to 6% (Delamare et al., 2020; Janda & Abbott, 2010). The bacteria is classified as an emerging pathogen, as its ability to cause food-borne illnesses through gastrointestinal infections, bacteraemia and septicemia was verified after its isolation (Fernández-Bravo & Figueras, 2020; Popoff & Véron, 1976). *A. hydrophila* belongs to the group of aerobic heterotrophic psychrotrophic bacteria (AHPB) characterized by the ability to grow at refrigeration temperatures, although its optimum growth temperature is mesophilic. They are associated immediately with the overall quality of refrigerated ready-to-eat foods, especially dairy products. The quantification of AHPB is used to measure the shelf life of shelf-stable foods (Oh & Lee, 2024), but it is not possible to say that their presence is directly correlated with pathogens such as *A. hydrophila*.

Beyond verifying their presence in food, various studies investigating the ability of pathogenic bacteria to develop through mathematical model have considered variations in the intrinsic and extrinsic conditions of food, with storage temperature being one of the most studied variables (Kim et al., 2022; Lee et al., 2023; Huang et al., 2023; Umutoni et al., 2020; Lee et al., 2022). Although its habitat is aquatic, its presence has been verified in different animal products (Abdulaal, 2019; Park et al., 2021; Patel et al., 2022; Silva et al., 2023) and plant products (Eluma et al., 2023; Teodoro et al., 2022; Umutoni et al., 2020). These studies suggest that its presence may be related the cross-contamination, contaminated raw materials, use of contaminated water during food production and preparation, which makes inadequate temperatures during transport and storage ideal conditions for its development.

Rosario et al. (2024) presented studies involving the impact that the microorganisms *Staphylococcus aureus*, *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli* can have on consumer health when present in different foods, including fresh cheeses, as well as the importance of using models for quality control.

Applications of regression equations with predictive and/or predictable data close to actual microbial count values can be a useful tool for the food industry when choosing the right final product distribution system, being able to accurately predict the rate of bacterial growth, especially in cases of land transportation, where thermal control is not always guaranteed and temperature fluctuations are common (Adams et al., 2024; Wu & Hsiao, 2021; Tang et al., 2021). The use of new technologies to prevent bacterial growth in ready-to-eat foods has been verified by Pei et al. (2023) who suggest the addition of natural antimicrobial compounds as agents to inhibit the growth of *A. hydrophila* in foods. In addition, Peron et al. (2022) emphasize the importance of using essential oils associated with active and intelligent packaging for food safety. Therefore, the use of models that can predict microbial development has been used in different areas of food quality, in an effort to reduce costs and ensure the safety of the food available on the consumer market.

Dairy products are pleasing to the palate, can provide essential nutrients for good development and are well accepted by different age groups (Novokshanova et al., 2023). “Minas Frescal” cheese stands out as a high-moisture fresh cheese, widely consumed in Brazil and of great economic importance (Silva et al., 2024). Known for its mild flavor and fresh aroma, this product is sold under refrigeration, which favors the development of psychrotrophic bacteria (Nájera et al., 2021; Silva et al., 2023). Therefore, monitoring the development of the microorganism by PCR techniques or the toxins produced in the food is extremely important to guarantee the quality of the product (Makkia et al., 2022).

Considering that *A. hydrophila* is a neglected bacterium and that there are no studies in the literature that specifically explore the prediction of the development of *A. hydrophila* in “Minas Frescal” cheese at domestic refrigeration temperatures (4, 8 and 12°C), it is possible that its ability to grow in fresh, nutrient-rich foods aids in the development of this microorganism. Therefore, research into the development of *Aeromonas* and AHPB is of fundamental importance in predicting the behavior of these microorganisms in ready-to-eat foods, such as “Minas Frescal” cheese.

The objective of this study was to determine the behavior of *A. hydrophila* in “Minas Frescal” cheese during storage at 4°C, 8°C and 12°C using a regression model and to predict the development of the microorganism over 24 days of storage.

2. Material and Method

For the investigation, the storage temperatures of “Minas Frescal” cheese at 4°C, 8°C and 12°C, which were measured using a thermo-hygrometer and did not vary by more than $\pm 1^\circ\text{C}$, were considered as the treatment. The samples were analyzed in triplicate during the shelf-life, for both microbiological and physicochemical parameters.

2.1 Preparation of Bacterial Solution

The inoculum was prepared from the *A. hydrophila* culture was isolated in our laboratory and identified using the Polymerase Chain Reaction (PCR) technique, targeting the 16S rRNA gene, using the species-specific primers Forward GAAAGGTTGATGCCTAATACGTA and Reverse CGTGCTGGCAACAAAGGACAG, with a size of 625 bp, following the identification method applied by Martins et al. (2023), stored on tryptic soy agar (TSA) under refrigeration (2°C), called the stock culture. The stock culture was pre-cultured aerobically on TSA and incubated at 28°C for 24 hours to obtain bacterial cells.

The fresh culture was suspended in 5mL of sterilized saline solution until it reached a turbidity equivalent to 0.5 on the McFarland scale (Lennette et al., 1985), resulting in a final concentration of approximately 5.83 log CFU in each mL of milk used to make “Minas Frescal” cheese. This initial bacterial concentration was chosen to ensure that the *Aeromonas* spp. contamination remained in the final product (cheese) until the shelf-life analysis was completed, as part of the initial contamination could be lost during the processing stages (whey drainage, for example). The use of this high level of contamination was decided as the worst possible condition; a lower level of contamination would have resulted in early inactivation of the pathogen, not allowing observation of the behavior of the microorganism throughout the storage period.

2.2 Production of “Minas Frescal” Cheese and Bacterial Inoculation

“Minas Frescal” cheese was prepared according to Campagnollo et al. (2018) with modifications. The pasteurized milk was heated to 35°C, followed by the addition of sodium chloride, 85% lactic acid, calcium chloride and rennet; after homogenization, 75mL of the prepared inoculum was added per liter of milk. Once the curd was obtained, it was homogenized for one hour and then the mass was transferred to appropriate cheese moulds, which were kept refrigerated for eight hours for desorption.

A total of 144 samples of “Minas Frescal” cheese, each weighing approximately 100g, were individually vacuum-packed. Cheese samples were stored in a refrigerator monitored at $4 \pm 1^\circ\text{C}$ (48 samples), at $8 \pm 1^\circ\text{C}$ (48 samples),

and at $12 \pm 1^\circ\text{C}$ (48 samples), simulating the limit temperature and abuse temperature allowed by current Brazilian legislation (Brazil, 1997).

2.3 *Aeromonas hydrophila* Quantification

The experiment was carried out over 24 days, simulating the shelf life of “Minas Frescal” cheese, with the analysis starting 24 hours after the product was made, both temperatures were analyzed on the same days and carried out in triplicate. The frequency of analysis and the decimal dilutions used for inoculation varied according to the counts obtained, with the aim of obtaining plates with counts between ten and 300 colony-forming units.

Quantification of *Aeromonas* spp. was carried out according to the methodology proposed by Rall et al. (1998), using peptone saline for serial decimal dilutions and the surface plating technique on amido ampicillin agar developed by Palumbo et al. (1985). The plates were read after 24 ± 2 hours of incubation at $28 \pm 1^\circ\text{C}$.

For biochemical confirmation, five colonies were selected from two different dilutions of each sample, purified in TSA, and subjected to the catalase reaction, oxidase reaction, three-sugar and iron agar reaction, and esculin hydrolysis according to the methods of Popoff and Véron (1976), Abbott et al. (2003) and Beaz-Hidalgo et al. (2013).

The frequency of analysis was at the same intervals as the quantification *A. hydrophila*, and the decimal dilutions used for inoculation varied according to the counts obtained.

Quantification of aerobic heterotrophic psychrotrophic bacteria (AHPB) was carried out according to the methodology proposed by American Public Health Association (APHA, 2015) using peptone water for serial decimal dilutions and the surface plating technique on plate count agar. The plates were read after seven days of incubation at $7 \pm 1^\circ\text{C}$.

2.4 Development Prevision *A. hydrophila*

The mean counts of *A. hydrophila* were plotted against storage time to create a scatter plot, with the objective of identifying the equation that best fits the data. As the coefficients of determination for the three storage temperatures analyzed were greater than or equal to 60%, the polynomial equation of degree two was selected for the purpose of predicting the development of *A. hydrophila*.

The results of the bacterial count averages were subjected to both an analysis of variance (ANOVA). In consideration of a significance level of 95%, the counts of *A. hydrophila* at 12°C were excluded from the subsequent analysis. The coefficients were then applied to the second-degree polynomial equation (Eq. 1).

$$y = A + Bx + Cx^2 \quad (1)$$

The coefficients can be interpreted to obtain the initial bacterial count (A), the bacterial growth rate (B) and the bacterial decline rate (C), taking into account the storage time (x). The prediction of the *A. hydrophila* count at each storage temperature, at 4°C (t_1) and 8°C (t_2) was determined by using the equation.

2.5 Water Activity and Potential Hydrogenionic Analysis

Water activity (wa) was determined using a water activity meter (AQUALAB model 4TE) and potential hydrogenionic (pH) using a previously calibrated tabletop pH meter (ALFSKIT model AT355). The analyses were carried out in triplicate during the storage of the “Minas Frescal” cheese, at the same intervals as the microbiological analysis, for 24 days, starting 24 hours after the product was made; both temperatures were analyzed on the same days and carried out in triplicate.

2.6 Statistical Analysis

Analysis of variance (ANOVA) was carried out on the pH and wa data for each time period and temperature independently. If no statistical difference was observed ($p < 0.05$) then the data was presented as mean values \pm standard deviation (descriptive analysis). For growth prediction, for each temperature, the data obtained was submitted to ANOVA for both *A. hydrophila* and AHPB, considering the second-degree polynomial equation, with significance $p < 0.05$. Pearson's correlation ($p < 0.05$) was applied to the quantification of *A. hydrophila* (log CFU/g), quantification of AHPB (log UFC/g) and pH of “Minas Frescal” cheese in the xlstat software version 2024.

3. Results and Discussion

Figure 1 shows the development curve of *A. hydrophila* and aerobic heterotrophic psychrotrophic bacteria (AHPB) in “Minas Frescal” cheese during 24 days of refrigerated storage at 4°C , 8°C and 12°C .

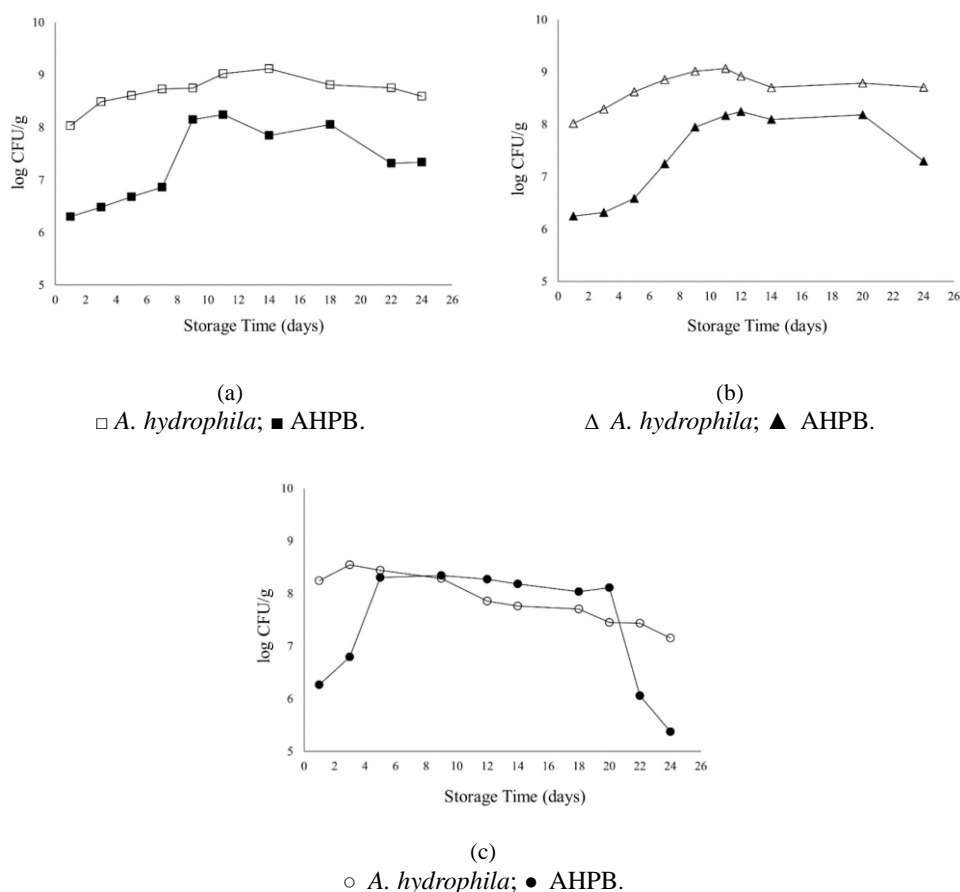


Figure 1. Growth curve (log CFU/g) of *Aeromonas hydrophila* and psychotrophic bacteria in “Minas Frescal” cheese during 24 days: (a) at 4°C; (b) at 8°C; (c) at 12°C

The development of *A. hydrophila* and AHPB was confirmed at all temperatures analyzed, corroborating the findings of Possas et al. (2021). These authors observed that fresh cheeses, such as “Minas Frescal”, facilitate bacterial growth during the product's shelf life by providing proteins, carbohydrates, and vitamins.

Were the amount of nutrients, and other intrinsic factors, such as water activity (wa) and potential hydrogen (pH), and extrinsic factors, such as temperature and the gas composition in which the matrix is inserted or packaged, have been identified by Fernández-Bravo and Figueras (2020) as determinants influencing bacterial development, particularly in the case of *Aeromonas*.

This research showed that storage at lower temperatures, such as 4°C and 8°C, allowed the development of *A. hydrophila* and AHPB, which showed similar characteristics (Figure 1a, 1b) as described by Tarlak and Pérez-Rodríguez (2021) for bacterial growth curves, in the well-defined adaptation, logarithmic, stationary and death stages. However, it can be seen that at 12 days the temperature of 12°C showed lower count values (7.86 log CFU/day) in contrast to storage at 8°C (8.92 log CFU/day) and 4°C (8.98 log CFU/day) (Figure 1a). Possibly the growth of other microorganisms such as lactic acid bacteria, common in dairy products, or psychrotrophic microorganisms affected the development of *Aeromonas* at 12°C, as reported by Fidan et al. (2022), products made from pasteurized milk allowed the growth of other microorganisms due to the inherent nature as high presence of moisture and nutrients, which reinforces the care in storage temperature.

The regression equations (2) and (3) were obtained from the mean values of *A. hydrophila* counts at temperatures of 4°C (t_1) and 8°C (t_2). The temperature parameters were highly significant ($p < 0.001$) at the storage temperature of “Minas Frescal” cheese at 4°C, where the *Aeromonas* development data was very similar to the equation of the regression model (Eq. 2) (R^2 : 0.90). The same second-degree linear regression model did not fit the observed data for *A. hydrophila* growth (Eq. 3).

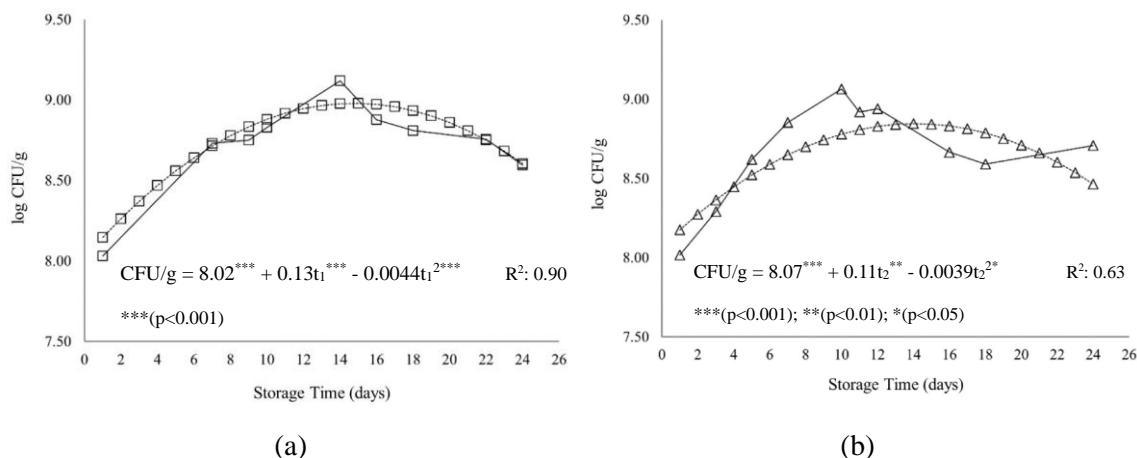
$$\text{CFU/g} = 8.02^{***} + 0.13t_1^{***} - 0.0044t_1^{2***} \quad R^2: 0.90 \quad (2)$$

$$\text{CFU/g} = 8.07^{***} + 0.11t_2^{**} - 0.0039t_2^{2*} \quad R^2: 0.63 \quad (3)$$

***($p < 0.001$); **($p < 0.01$); *($p < 0.05$)

The growth rate of *A. hydrophila*, 0.13 log CFU/day, demonstrates that the temperature of 4°C is the most suitable for the development of this microorganism, in contrast to that verified for 8°C (0.11 log CFU/day), as well as the rate of decline 0.0044 log CFU/day and 0.0039 log CFU/day, respectively 4°C and 8°C.

The regression model (Figure 2) was able to predict the *Aeromonas* count perfectly up to 12 days of storage at 4°C, predicting a count of 8.95 log CFU/day. Yang, Park and Ha (2016) demonstrated this preference for refrigeration temperature by *A. hydrophila* which suggests that, in order to inhibit the development of this microorganism at refrigeration temperatures, other parameters such as pH and sodium chloride content should be considered.



(-) average count values obtained; (···) values predicted by the regression model. (a) *Aeromonas hydrophila* at 4°C; (b) psychrotrophic bacteria. Figure 2. Growth curve (log CFU/g) of *Aeromonas hydrophila* at 4°C (a) and psychrotrophic bacteria (b) in “Minas Frescal” cheese during 24 days. ***($p < 0.001$); **($p < 0.01$); *($p < 0.05$)

A. hydrophila does not tolerate a pH lower than 6 or a salt content above 7% (Daskalov, 2006; Fernández-Bravo & Figueras, 2020; Kirov, 1993). During storage, the pH values remained above 6.5 (Table 1), and the salt concentration used to make the product was 1.5%, which shows that temperature was the limiting factor for the bacteria's development.

Table 1. Mean \pm standard deviation of potential hydrogenionic (pH) and water activity (wa) readings were recorded over a period of storage of “Minas Frescal” cheese in days at 4°C and 8°C

Storage Time (days)	pH		wa	
	4°C	8°C	4°C	8°C
1	6.63 \pm 0.04	6.65 \pm 0.03	0.9925 \pm 0.0027	0.9945 \pm 0.0017
5	6.82 \pm 0.00	6.85 \pm 0.03	0.9873 \pm 0.0005	0.9856 \pm 0.0001
7	6.96 \pm 0.20	6.85 \pm 0.13	0.9951 \pm 0.0001	0.9886 \pm 0.0052
9	6.99 \pm 0.01	7.04 \pm 0.01	0.9919 \pm 0.0008	0.9946 \pm 0.0014
10	6.79 \pm 0.01	6.79 \pm 0.00	0.9898 \pm 0.0036	0.9929 \pm 0.0016
12	7.03 \pm 0.00	7.02 \pm 0.01	0.9863 \pm 0.0029	0.9820 \pm 0.0020
14	6.97 \pm 0.00	7.01 \pm 0.01	0.9919 \pm 0.0005	0.9847 \pm 0.0015
16	6.97 \pm 0.00	6.98 \pm 0.03	0.9893 \pm 0.0002	0.9872 \pm 0.0033
18	6.87 \pm 0.16	7.04 \pm 0.04	0.9869 \pm 0.0002	0.9891 \pm 0.0021
20	7.01 \pm 0.00	7.02 \pm 0.00	0.9831 \pm 0.0005	0.9837 \pm 0.0029
22	6.75 \pm 0.01	7.05 \pm 0.01	0.9890 \pm 0.0005	0.9882 \pm 0.0008
24	7.04 \pm 0.02	7.07 \pm 0.00	0.9860 \pm 0.0002	0.9859 \pm 0.0013

A preference for refrigeration temperatures by *A. hydrophila*, which is classified as a psychrotrophic bacteria, can be explained by the greater availability of nutrients when other microorganisms are present in the environment,

since lower temperatures induce a slower metabolism in many bacteria, which, if they were to compete in the environment, could hinder the development of *Aeromonas* (Rabêlo et al., 2021).

For the food industry, the use of tools such as bacterial quantification prediction equations can help to predict, from the initial count, the level of count in storage periods, especially for this type of dairy product where there is high water activity, pH above 6.5 (Table 1) and low salt content (1.5%), excellent conditions for the development of *Aeromonas*. Such conditions of storage temperature, pH and high water activity act as an ideal environment for microbial development and chemical and enzymatic reactions, ensuring the survival and development of *A. hydrophila* through the supply of energy (Guan & Lui, 2020; Vasuki, Kadirvel, Narayana, 2023).

Based on the model obtained (Figure 2a), the predicted *A. hydrophila* count values were close to the actual counts until the 13th day. Although low temperatures allow the development of this microorganism, they reduce oxidation and proteolysis reactions, influencing product quality during the refrigerated shelf life (Gao et al., 2020), and the model obtained makes it possible to predict the maintenance of product quality up to 13 days, for a storage temperature of 4°C.

During the storage time of “Minas Frescal” cheese, there was a slight increase in the product's pH at the temperatures analyzed, but little variation in water activity (wa). Variations in the conditions of the development medium, such as acidification, are regulated by the bacteria's intracellular metabolism through the physical barrier of the cell wall and outer membrane protein, allowing interactions between multiple organelle transport systems and increasing development tolerance (Begley & Hill, 2015; Mitchell & Silhavy, 2019).

Table 2 shows the strong correlation between AHPB and *A. hydrophila*, demonstrating that although *Aeromonas* had ideal conditions in the environment to grow, there was competition between other microorganisms present, with similar temperature and pH requirements. The observed positive and highly significant Pearson's correlation (R: 0.762, $p < 0.001$) for the 4°C temperature (Table 2) is compared with the behavior of AHPB shown in Figure 1, in which the *A. hydrophila* count shows an increase in the development curve, except for the 12°C temperature, until around 10 days. This demonstrates that monitoring pH does not indicate the microbiological quality of the cheese with regard to the development of *Aeromonas*.

Table 2. Correlation by Person's R between *Aeromonas hydrophila* quantification (log CFU/g), aerobic heterotrophic psychrotrophic bacteria (AHPB) quantification (log CFU/g) and potential hydrogenionic (pH) of “Minas Frescal” cheese during storage at 4°C and 8°C

	AHPB 4°C	AHPB 8°C	pH 4°C	pH 8°C
<i>A. hydrophila</i> 4°C	0.762***	-	0.628**	-
<i>A. hydrophila</i> 8°C	-	0.598*	-	0.682**
AHPB 4°C	-	-	0.714**	-
AHPB 8°C	-	-	-	0.556*
pH 4°C	-	-	-	-

- No correlation *** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05

Lee et al. (2023) observed that processing conditions had little influence on the development of *Aeromonas* spp. This was also observed in this study, in which pH and wa did not influence the development of *A. hydrophila*. The use of new technologies to inhibit bacterial growth in ready-to-eat foods has been verified. Pei et al. (2023) suggests the addition of natural antimicrobial compounds as agents to inhibit the development of *A. hydrophila* in foods. In the specific case of *A. hydrophila*, in “Minas Frescal” cheese, the temperature of 4°C stimulated the development of the microorganism.

“Minas Frescal” cheese is a fresh food with nutrients that favored the development of AHPB at all the storage temperatures analyzed. However, the temperature of 12°C hindered the development of *A. hydrophila*, though it had no impact on the AHPB present.

The model obtained for *Aeromonas hydrophila* fitted better at a temperature of 4°C, allowing greater control over the development of the microorganism, with the prediction fitting the model up to the 13th day. The results obtained in this study can be used as a basis for developing new control strategies and predicting the development of *A. hydrophila* in the dairy industry, specifically in “Minas Frescal” cheese, contributing to improving food safety and public health.

Although temperatures above 4°C hinder the growth of *A. hydrophila*, other microorganisms could grow in the product, given that the pH and water activity would be optimal for this. Pasteurization could be applied to the

product after filling, however future studies would be required to investigate whether the procedure on cheeses considered “fresh cheeses” would not affect the sensory quality of the final product. Alternative food safety methods such as hazard analysis critical control points (HACCP), the use of active, modified packaging or controlled atmosphere packaging should be investigated to assess the development of *A. hydrophila* in dairy products.

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Authors contributions

Prof. D.O. Ritter and Prof. M. Lanzarin were responsible for study design and revising. MSc in Food Science and Technology H. C. L. Winter was responsible for data collection. Prof. R.A.P.G. Faria and Prof. E. Nascimento drafted the manuscript and Prof. M. Lanzarin revised it. Students M. F. S. Rodrigues and I. A. Silva contributed equally to the study. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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References

- Abbott, S. L., Cheung, W. K. W., & Janda, J. M. (2003). The Genus *Aeromonas*: Biochemical characteristics, atypical reactions, and phenotypic identification schemes. *J. Clin. Microbiol.*, 41, 2348-2357. <https://doi.org/10.1128/jcm.41.6.2348-2357.2003>
- Abdulaal, N. I. (2019). Detection of *Aeromonas hydrophila* in raw milk and soft cheese in Baghdad city. *Iraqi J. Vet. Med.*, 43, 52-60. <https://doi.org/10.30539/iraqijvm.v43i2.531>
- Adams, M. R., McClure, P. J., & Moss, M. (2024). *Food Microbiology*. In *Royal Society of Chemistry* (5th ed.). pp. 5582. <https://doi.org/10.1039/9781837673698>

- Beagly, M., & Hill, C. (2015). Stress adaptation in foodborne pathogens. *Annu. Rev. Food Sci. Technol.*, 6, 191-210. <https://doi.org/10.1146/annurev-food-030713-092350>
- Beaz-Hidalgo, R., Martínez-Murcia, A., & Figueras, M. J. (2013). Reclassification of *Aeromonas hydrophila* subsp. *dhakensis* Huys et al. 2002 and *Aeromonas aquariorum* Martínez-Murcia et al. 2008 as *Aeromonas dhakensis* sp. nov. comb. nov. and emendation of the species *Aeromonas hydrophila*. *Syst. Appl. Microbiol.*, 36, 171-176. <https://doi.org/10.1016/j.syapm.2014.08.001>
- Brazil. (1997). *Portaria nº 352 de 4 de setembro de 1997*. Approves the Technical Regulation for Establishing the Identity and Quality of Minas Frescal Cheese. Retrieved from <https://www.gov.br/agricultura/pt-br/assuntos/defesa-agropecuaria/suasa/regulamentos-tecnicos-de-identidade-e-qualidade-de-produtos-de-origem-animal-1/rtiq-leite-e-seus-derivados>
- Campagnollo, F. B., Margalho, L. P., Kamimura, B. A., Feliciano, M. D., Freire, L., ... Sant'ana, A. S. (2018). Selection of indigenous lactic acid bacteria presenting anti-listerial activity, and their role in reducing the maturation period and assuring the safety of traditional brazilian cheeses. *Food Microbiol.*, 73, 288-297. <https://doi.org/10.1016/j.fm.2018.02.006>
- Daskalov, H. (2006). The importance of *Aeromonas hydrophila* in food safety. *Food Control*, 17, 474-83. <https://doi.org/10.1016/j.foodcont.2005.02.009>
- Delamare, A. P. L., Costa, S. O. P., Silveira, M. M., & Echeverrigaray, S. (2020). Growth of *Aeromonas* species on increasing concentrations of sodium chloride. *Lett. Appl. Microbiol.*, 30, 57-60. <https://doi.org/10.1046/j.1472-765x.2000.00662.x>
- Eluma, M., Itelima, J. U., Onwuliri, F. C., Darda, F. O., & Moses, J. D. (2023). The prevalence of *Aeromonas* species in salad vegetables sourced from four local government areas of plateau state. *Int. J. Pharm. Bio. Scie.*, 3, 191-197, <https://doi.org/10.47191/ijpbms/v3-i5-04>
- Fernández-Bravo, A., & Figueras, M. J. (2020). An update on the genus *Aeromonas*: Taxonomy, epidemiology, and pathogenicity. *Microorganisms*, 8, 3-6. <https://doi.org/10.3390/microorganisms8010129>
- Fidan, H., Esatbeyoglu, T., Simat, V., Trif, M., Tabanelli, G., ... Özogul, F. (2022). Recent developments of lactic acid bacteria and their metabolites on foodborne pathogens and spoilage bacteria: facts and gaps. *Food Biosci.*, 47, 101741. <https://doi.org/10.1016/j.fbio.2022.101741>
- Guan, N., & Liu, L. (2020). Microbial response to acid stress: mechanisms and applications. *Appl. Microbiol. Biotechnol.*, 104, 51-65. <https://doi.org/10.1007/s00253-019-10226-1>
- Huang, W., Wang, X., Zhang, J., Xia, J., & Zhang, X. (2023). Improvement of blueberry freshness prediction based on machine learning and multi-source sensing in the cold chain logistics. *Food Control*, 145, 109496. <https://doi.org/10.1016/j.foodcont.2022.109496>
- Janda, J. M., & Abbott, S. L. (2010). The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clin. Microbiol. Rev.*, 23, 35-73. <https://doi.org/10.1128/CMR.00039-09>
- Kim, J. Y., Jeon, E. B., Song, M. G., Park, S. H., & Park, S. Y. (2022). Development of predictive growth models of *Aeromonas hydrophila* on raw tuna *Thunnus orientalis* as a function of storage temperatures. *Food Sci. Tech.*, 156, 113052. <https://doi.org/10.1016/j.lwt.2021.113052>
- Kirov S. M. (1993). The public health significance of *Aeromonas* spp. in foods. *Int. J. Food Microbiol.*, 20, 179-198. [https://doi.org/10.1016/0168-1605\(93\)90164-c](https://doi.org/10.1016/0168-1605(93)90164-c)
- Lee, H., Tokle, I. F., Lunestad, B., Lerfall, J., Hoel, S., & Jakobsen, A. N. (2023). The effect of food processing factors on the growth kinetics of *Aeromonas* strains isolated from ready-to-eat seafood. *Int. J. Food Microbiol.*, 384, 109985. <https://doi.org/10.1016/j.ijfoodmicro.2022.109985>
- Lee, S., Han, A., Yoon, J., & Lee, S. (2022). Growth evaluation of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* in fresh fruit and vegetable juices via predictive modeling. *LWT*, 162, 1-5. <https://doi.org/10.1016/j.lwt.2022.113485>
- Lenette, E. H., Balows, A., Hausler, W. J., & Shadomy, H. J. (1985). *Manual of Clinical Microbiology*. American Society for Microbiology, 4th ed., American Association for Microbiology, Washington DC.
- Makkia, D. I., Mahmoud, A. H., Bahout, A. A., Bayoumi, M. A., & Alnakip, M. E. (2022). Molecular Studies on Some Emerging Pathogens in Dairy Products Retailed in Dakahlia Governorate, Egypt. *J. Adv. Vet. Res.*, 12, 392-398.

- Martins, D. L., Cabral, A. N., Winter, H. C. L., Mariotto, S., Nascimento, E., ... Lanzarin, M. (2023). Resistance of *Aeromonas hydrophila* isolates to antimicrobials and sanitizers. *Cien. Rural*, 53, 1-9. <https://doi.org/10.1590/0103-8478cr20220256>
- Mitchell, A. M., & Silhavy, T. J. (2019). Envelope stress responses: balancing damage repair and toxicity. *Nat Rev Microbiol.*, 17, 417-428. <https://doi.org/10.1038/s41579-019-0199-0>
- Nájera, A. I., Nieto, S., Barron, L. J. R., & Albisu, M. (2021). A Review of the preservation of hard and semi-hard cheeses: quality and safety. *Int. J. Environ. Res. Public Health.*, 18, 9789. <https://doi.org/10.3390/ijerph18189789>
- Novokshanova, A. L., Matveeva, N. O., & Nikityuk, D. B. (2024). Analysis of milk consumption and dairy products of the Russian population using an online survey. *Food Sci Nutr.*, 12, 933-942. <https://doi.org/10.1002/fsn3.3808>
- Oh, H., & Lee, J. (2024). Psychrotrophic bacteria threatening the safety of animal-derived foods: characteristics, contamination, and control strategies. *Food Sci. Anim. Resour.*, 44, 1011-27. <https://doi.org/10.5851/kosfa.2024.e70>
- Palumbo, S. A., Maxino, F., Williams, A. C., Buchanan, R. L., & Thayer, D. W. (1985). Starch ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. *Appl. Environ. Microbiol.*, 50, 1027-1030. <https://doi.org/10.1128/aem.50.4.1027-1030.1985>
- Park, S. M., Kim, H. W., Choi, C., & Rhee, M. S. (2021). Pathogenicity and seasonal variation of *Aeromonas hydrophila* isolated from seafood and ready-to-eat Sushi in South Korea. *Food Res. Int.*, 147, 110484. <https://doi.org/10.1016/j.foodres.2021.110484>
- Patel, K. J., Kumar, R., Savalia, C. V., Nayak, D. N., & Kalyani, I. H. (2022). Determination of prevalence and multidrug resistant *Aeromonas* in raw milk from dairy animals. *J. Pharm. Innov.*, 12, 1120-1125.
- Pei, J., Cunxin, S., Liu, B., Zhou, Q., Zheng, X., Liu, B., Zhao, C., & Sun, C. (2023). Study of antibacterial properties of cinnamaldehyde against *Aeromonas hydrophila*. *Aquac. Res.*, 2023, 1-11. <https://doi.org/10.1155/2023/1191123>
- Peron, T., Santos, T. C. C., Silva, L. D. S., Arruda, T. R., & Leite Júnior, B. R. C. (2022). Active Packaging: An alternative to minimum processed vegetables? *Res. Soc. Dev.*, 11, 1-12. <https://doi.org/10.33448/rsd-v11i10.33043>
- Popoff, M., & Verón, M. (1976). A taxonomic study of the *Aeromonas hydrophila* - *Aeromonas punctata* group. *J. Gen. Microbiol.*, 94, 11-22. <https://doi.org/10.1099/00221287-94-1-11>
- Possas A., Bonilla-Luque, O. M., & Valero, A. (2021). From cheese-making to consumption: exploring the microbial safety of cheeses through predictive microbiology models. *Foods.*, 10, 1-22. <https://doi.org/10.3390/foods10020355>
- Rabêlo, C. A., Ricardo, M., Porfírio, J. A., Pimentel, T. C., Nascimento, J. S., & Costa L. E. O. (2021). Psychrotrophic bacteria in brazilian organic dairy products: identification, production of deteriorating enzymes and biofilm formation. *Food Sci. and Tech.*, 41, 799-806. <https://doi.org/10.1590/fst.68420>
- Rall, V. L. M., Iaria, S. T., Heidtmann, S., Pimenta, F. C., Gamba, R. C., & Pedroso, D. M. M. (1998). *Aeromonas* species isolated from pintado fish (*Pseudoplatystoma* sp): virulence factors and drug susceptibility. *Food Microbiol.*, 29, 222-227. <https://doi.org/10.1590/S0001-37141998000300015>
- Rosario, I. L. S., Pia, A. K. R., Rekowsky, B. S. S., Elias, S. O., Noronha, T. B., ... Conte-Junior, C. A. (2024). Predictive model for the growth of Shiga toxin-producing *Escherichia coli* in Minas Frescal cheese. *Microb. Risk Anal.*, 27-28, 100308. <https://doi.org/10.1016/j.mran.2024.100308>
- Silva, A. A., Leite, J. N., Winter, H. C. L., Furtado, T. L. J., Morais, N. M. L., ... Lanzarin, M. (2023). *Aeromonas* sp. in freshwater fish and antimicrobial resistance: emerging pathogen. *Cienc. Rural*, 53, 1-14. <https://doi.org/10.1590/0103-8478cr20220088>
- Silva, T. C. M., Ramos, G. L. P. A., Prudêncio, E. S., Pimentel, T. C., Martins, C. C., ... Cruz, A. G. (2024). Functional Minas Frescal cheese with spore-forming *Weizmannia coagulans* GBI-30. *Int. Dairy J.*, 156, 105993. <https://doi.org/10.1016/j.idairyj.2024.105993>
- Tang, J., Zou, Y., Xie, R., Tu, N., & Liu, G. (2021). Compact supervisory system for cold chain logistics. *Food Control*, 126, 108025. <https://doi.org/10.1016/j.foodcont.2021.108025>

- Tarlak, F., & Pérez-Rodríguez, F. (2021). Development and validation of a one-step modelling approach for the determination of chicken meat shelf-life based on the growth kinetics of *Pseudomonas* spp. *Food Scie. Technol. Int.*, 28, 672-682. <https://doi.org/10.1177/10820132211049616>
- Teodoro, J. R., Carvalho, G. G., Queiroz, M. M., Levy, C. E., & Kabuki, D. Y. (2022). Incidence, evaluation of detection and identification methods, and antimicrobial resistance of *Aeromonas* spp. in ready-to-eat foods. *Int. J. Food Microbiol.*, 379, 109862. <https://doi.org/10.1016/j.ijfoodmicro.2022.109862>
- Umutoni, N., Jakobsen, A. N., Mukhatov, K., Thomassen, G. M. B., Karlsen, H., & Mhli, L. (2020). Occurrence, diversity and temperature-dependent growth kinetics of *Aeromonas* spp. in lettuce. *Int. J. Food Microbiol.*, 335, 108852. <https://doi.org/10.1016/j.ijfoodmicro.2020.108852>
- Vasuki, M. T., Kadirvel, V., & Narayana, G. P. (2023). Smart packaging: An overview of concepts and applications in various food industries. *Food Bioeng.*, 2, 25-41. <https://doi.org/10.1002/fbe2.12038>
- Wu, J., & Hsiao, H. (2021). Food quality and safety risk diagnosis in the food cold chain through failure mode and effect analysis. *Food Control*, 120, 107501. <https://doi.org/10.1016/j.foodcont.2020.107501>
- Yang, S., Park, S. Y., & Ha, S. (2016). A Predictive growth model of *Aeromonas hydrophila* on chicken breasts under various storage temperatures. *Food Sci. Technol.*, 69, 98-103. <https://doi.org/10.1016/j.lwt.2016.01.016>