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## Exploring the Impact of the Matrix on the Growth of *Listeria monocytogenes*

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### Abstract

Listeriosis is a significant foodborne illness mainly resulting from the consumption of food contaminated with *Listeria monocytogenes*. The present research aimed to comprehensively examine the growth of foodborne pathogen *Listeria monocytogenes* in readily accessible ultra-high temperature processed bovine milk, infant formula, and two commercial plant-based beverages (almond and coconut) stored at ambient temperature (25°C). Nutrient broth was used as a reference control. Beverage samples were inoculated with *Listeria monocytogenes* at approximately  $9 \times 10^7$  CFU/mL. The inoculated samples were stored at 25°C and sampled on the 1, 3, 6, 24, 48 and 72 hour of incubation. The findings indicate that *Listeria monocytogenes* was capable of proliferating in all the samples reaching the highest counts on the 48<sup>th</sup> hour of the evaluation. Although that on the 48<sup>th</sup> hour of evaluation, coconut beverage had the lowest bacteria counts ( $7.5 \pm 0.98$  CFU/mL), no statistically important differences ( $P > 0.05$ ) in growth rates between diverse evaluated beverages was noted. At the 72-hour incubation period, infant formula exhibited the highest microbial counts, at a value of 8 logs, whereas the coconut beverage showed the lowest microbial counts at 6 logs. The obtained data indicate that all the assessed beverages could pose a considerable threat for listeriosis, and post-opening guidelines should be thoroughly evaluated.

**Keywords:** Bovine milk, Plant-based beverages, Infant formula, *Listeria monocytogenes*

### Introduction

Milk is a biologically complex fluid with a distinctive composition that supports the growth and development of newborns. It contains a variety of bioactive compounds known to offer health benefits (Ballard et al., 2013). However, milk also serves as a significant reservoir for foodborne pathogens, including *Listeria monocytogenes* (Erol and Taşçı, 2021; Kahraman et al., 2024; Rugji and Dinçoğlu, 2022; Rugji et al., 2023). *Listeria monocytogenes* is a pathogenic microorganism responsible for foodborne illnesses, and its capacity to endure and potentially exponentiate under a variety of adverse conditions presents a substantial food safety concern (Wang et al., 2024). Although listeriosis remains relatively rare, it can lead to severe, life-threatening infections in vulnerable populations, including infants, pregnant women, the elderly, and individuals with weakened immune systems (Pal et al., 2022).

In recent years, the growing popularity of vegan diets, increasing awareness of lactose intolerance, and heightened environmental consciousness have fueled the demand for plant-based alternatives to cow's milk (Bartula et al., 2023). There has been reported that in Europe, sales increased by 49% from 2018 to 2020, reaching €3.6 billion. According to the Smart Protein report, plant-based milk leads the plant-based product market in 8 of the 11 countries studied (Anon, 2021). While commercially available plant-based beverages are typically heat-treated to ensure microbiological safety, risks persist, such as the potential for cross-contamination post-processing or the survival of spores from spore-forming microorganisms despite ultra-high temperature (UHT) treatment (Bartula et al., 2023). The nutrient-dense composition and high-water activity of these beverages provide an environment conducive to the growth and spread of *L. monocytogenes*, presenting potential food safety hazards.

This study explores how different matrices—plant-based milk alternatives, bovine milk, infant formula, and culture media—affect the growth of *Listeria monocytogenes* when stored at 25 °C.

### Materials and Methods

#### Sources of Samples

The current dataset included four varieties of non-refrigerated UHT samples: whole bovine milk (Migros), almond (Alpro), Coconut (Alpro), Infant formula (Aptamil). Nutrient broth was used as reference control. All the samples were purchased from local store and for consistency, one brand for each beverage was selected for all experiments. Product information is provided in Table 1.



Table 1. UHT beverage composition.

100 g/mL	Bovine milk	Infant formula	Almond beverage	Coconut beverage
Fat	3.1	3.4	1.1	1.2
Carbohydrate	4.7	7.4	3.0	0.0
Protein	3	1.3	0.5	0.1
Salts	0.1	0.0	0.14	0.07

### **Bacterial Strain and Inoculum Preparation**

*Listeria monocytogenes* ATCC 19115 was used in the current investigation. A loop from each cryogenic (-80°C) stock vial was initially cultured individually in nutrient broth and further maintained on trypticase soy agar (TSA) at 4 °C throughout the study. One isolated colony was transferred to 9 mL of nutrient broth and incubated at 37°C for 24 h. Culture was then sub-cultured onto TSA and incubated at 37°C for 24 h. Following incubation, the microorganism was adjusted to a 0.5 McFarland standard in 9 mL of saline solution. Serial 2-fold dilutions were prepared on saline solution to determine the initial bacterial load.

### **Experimental Design**

*Listeria monocytogenes* survival was evaluated in five different matrices at 25°C for 72h. The temperature selection for this investigation was based on conditions designed to replicate ambient environments, simulating how inoculated bacteria would behave under post-thermal treatment contamination in the selected beverages. A total of 200 mL sample (Rugji et al., 2023) was inoculated with 1 mL of the prepared strain ( $9 \times 10^7$  CFU/mL). The inoculated samples were stored at 25°C and sampled on the 1, 3, 6, 24, 48 and 72 h of incubation. A total of 100 uL was sampled each time, was serially diluted and plated on TSA. The sampling times and total incubation were adjusted based on experimental feasibility. Experiments were conducted in two biologically independent replicates.

### **Data Analysis**

Results are presented as the mean value  $\pm$  standard deviation. Statistical analysis was conducted using GraphPad Prism version 10 (GraphPad Software Inc., La Jolla, CA, USA). P values  $\leq$  0.05 were regarded as statistically significant.

## **Results and Discussion**

The maximum growth rates of *Listeria monocytogenes* at ambient temperature (25°C) were assessed and compared across bovine milk, infant formula, and plant-based, non-dairy beverages. The *Listeria* strain (*Listeria monocytogenes* ATCC 19115) chosen for this study was a standard laboratory strain frequently utilized in food safety research. In a controlled setting, the microorganism demonstrated robust growth in all the tested matrices (Figure 1). However, no statistically significant difference ( $P > 0.05$ ) was found between the various types of beverages. The data from this study showed that *Listeria monocytogenes* was able to grow similarly in a selection of plant-based milk beverages (coconut, almond), infant formula and dairy milk, despite the significant differences in their compositions. An evaluation of bacterial counts 72 hours after incubation showed a numerical decrease; however, the levels remained high enough to pose a food safety risk (Table 2). At the 72-hour time point, the highest bacterial counts were observed in the infant formula, highlighting its significant relevance to food safety and preservation for this product category. While the counts in the plant-based beverages, almond and coconut, were lower at 6.1 and 6, respectively, these levels still represent a public health concern and should be regarded as a potential danger. Furthermore, the positive correlation values observed for the tested beverages indicate a direct relationship between the variables, suggesting that as one factor increases, the other tends to increase as well (Figure 2). The obtained data offer valuable insight into the comparable growth rate of *Listeria monocytogenes*, irrespective of the food matrix. Bartula et al. (2023) investigated the growth of various foodborne pathogens in commercial plant-based beverages and found that *Listeria* strains proliferated more rapidly in plant-based beverages than in bovine milk at both 8°C and 20°C. UHT beverages can be stored at ambient temperature for up to 12 months. Notably, the recommended post-opening storage duration for UHT bovine milk is 3 days under refrigeration, while UHT plant-based beverages can be stored for 5 days at refrigeration temperature. Thus, if these products become contaminated with *Listeria monocytogenes*, this study demonstrates that all can support rapid microbial growth if not stored at appropriate temperatures. Given that *Listeria monocytogenes* can be present on nuts and potentially enter products as a post-processing contaminant, the findings suggest that plant-based beverages may pose a considerable risk for listeriosis. Consequently, post-opening storage guidelines should be carefully reviewed in light of these results. The absence of a significant difference in growth rates between dairy and plant-based products for most of the microbes tested was unexpected, considering the differences in composition. Although plant-based beverages typically contain lower nutrient concentrations than cow's milk, they clearly supply sufficient nutrients to support similar growth rates (Bartula et al., 2023).



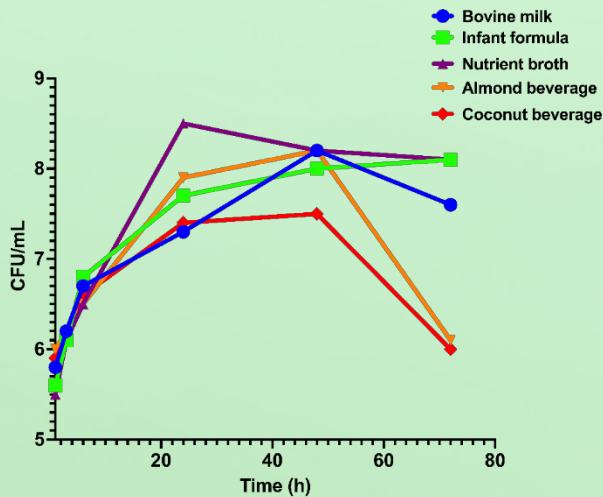


Figure 1. Growth curve of *Listeria monocytogenes* on selected beverages.

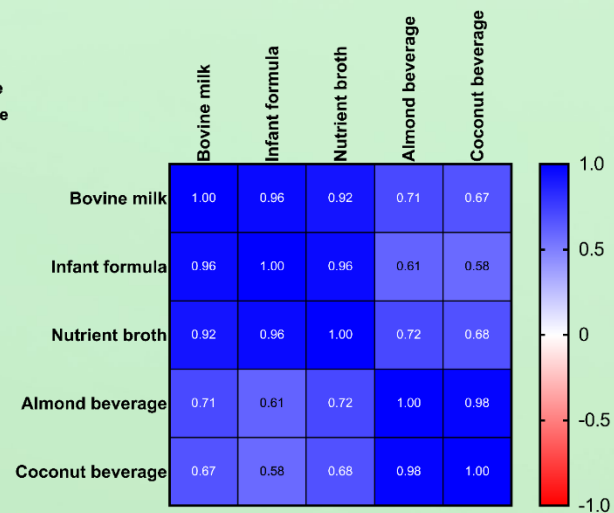


Figure 2. Pearson r correlation results.

Table 2. *Listeria monocytogenes* counts of the artificially contaminated beverages.

Time (h)	1	3	6	24	48	72
Bovine milk	5.8±0.02	6.2±0.00	6.7±0.03	7.3±0.38	8.2±0.11	7.6±0.80
Infant formula	5.6±0.19	6.1±0.17	6.8±0.02	7.7±0.76	8.0±0.49	8.1±0.87
Almond beverage	6.0±0.21	6.2±0.03	6.5±0.31	7.9±0.34	8.2±0.32	6.1±0.12
Coconut beverage	5.9±0.02	6.2±0.01	6.6±0.02	7.4±0.16	7.5±0.98	6.0±0.00
Nutrient broth	5.5±0.21	6.1±0.13	6.5±0.25	8.5±0.81	8.1±0.00	8.1±0.20

This study also emphasizes that the integration of food safety management systems and effective cleaning practices is crucial in the food industry to prevent contamination, ensure product safety, and maintain consumer health (Dinçoğlu and Rugji, 2021).

## Conclusion

Overall, the findings presented in this report offer critical insights into the growth dynamics of *Listeria monocytogenes*, a major foodborne pathogen, across diverse matrices, including the increasingly popular plant-based beverages gaining global traction. These findings must be taken into account when evaluating the risks linked to the production and consumption of plant-based milk alternatives.

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