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B.S. in Food Science and Technology



Special Graduation Project

**Evaluating the efficacy of mechanical removal of *Listeria monocytogenes*
from stainless steel and ceramic surfaces.**

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Abstract

This study conducted at Louisiana State University's Animal and Food Science Laboratory, investigated the detachment and bio-transfer of *Listeria monocytogenes* on stainless steel and ceramic surfaces, simulating food and non-food contact surfaces. An independent samples t-test design with 3 repetitions and 4 replicates each was used, employing mechanical action with glass beads to detach biofilms. After treatment, bacterial growth and transfer to fresh milk were assessed, simulating real-world contamination scenarios.

The *Listeria monocytogenes* V7 strain (milk-associated outbreak strain) was used to form biofilms on sterilized stainless steel and ceramic tiles. Mechanical action with glass beads in phosphate-buffered saline (PBS) and vortexing for two minutes each detached bacteria, followed by serial dilutions and plating for analysis.

Results indicated significantly higher biofilm detachment from stainless steel due to its lower porosity compared to ceramic. Mechanical force effectively reduced biofilm attachment, with bacterial transfer being lower within the first two minutes but significantly increasing by five minutes due to longer exposure.

This study emphasizes the importance of stringent cleaning protocols, combining mechanical action with disinfection to ensure pathogen removal, particularly on stainless steel. Minimizing contact time between surfaces and fresh foods is crucial for reducing cross-contamination risks, ensuring food safety, and maintaining hygiene standards in the dairy industry, particularly in preventing biofilm formation and bacterial spread.

Keywords: bio transference, dairy processing environments, food contact surfaces, non-food contact surfaces.

Resumen

Este estudio, realizado en la Universidad Estatal de Luisiana, investigó el desprendimiento y bio transferencia de *Listeria monocitógenes* en superficies de acero inoxidable y baldosas de cerámica, simulando superficies de contacto y no contacto con alimentos en una planta de procesamiento de lácteos. Se utilizó un diseño de muestras independientes y se analizó a través de una prueba t con 3 repeticiones de 4 réplicas cada una, empleando acción mecánica con esferas de vidrio para desprender biopelículas. Después del tratamiento, las muestras se incubaron para evaluar el crecimiento bacteriano y la transferencia a leche fresca.

La cepa de *Listeria* (V7 serotipo ½ a) fue obtenida y propagada bajo condiciones controladas. Los fragmentos de acero inoxidable y baldosas de cerámica fueron esterilizados y preparados para la formación de biopelículas. La extracción bacteriana involucró agitación mecánica con esferas de vidrio en solución salina tamponada con fosfatos (PBS), seguida de diluciones seriadas y cultivo en placa.

Los resultados indicaron un desprendimiento significativamente mayor de *Listeria* en superficies de acero inoxidable, atribuido a la rugosidad que mejora la adhesión bacteriana. La bio transferencia a la leche fue mínima en los primeros dos minutos, pero aumentó notablemente a los cinco minutos. La fuerza mecánica efectivamente elimina la biopelícula, es por esto por lo que en la bio transferencia se muestra una carga microbiana menor en los primeros dos minutos respectivamente.

Este estudio enfatiza la importancia de protocolos de limpieza rigurosos en combinación con la desinfección y acción mecánica en las superficies, especialmente para el acero inoxidable, para mitigar la contaminación bacteriana. Minimizar el tiempo de contacto entre superficies y alimentos frescos es crucial para reducir los riesgos de contaminación cruzada, mejorando la seguridad alimentaria en entornos de procesamiento de lácteos. Los hallazgos informan las mejores prácticas para mantener estándares de higiene y seguridad en la industria láctea.

Palabras clave: área de contacto con alimentos, áreas de no contacto con alimentos, áreas de procesamiento de productos lácteos, bio transferencia.

Introduction

Listeria monocytogenes (Lm) is a gram-positive, rod-shaped bacteria responsible for human listeriosis by consuming contaminated food (Orsi et al., 2011). This microorganism is a major concern for the food industry since it is the causal agent of listeriosis, a severe disease with high hospitalization and case-fatality rates approximately of 94% and 25% respectively (Rohilla et al., 2024). Human listeriosis ranges from subclinical and uncomplicated febrile gastro-enteritis to severe invasive disease (Bortolussi, 2008). *Listeria monocytogenes* infection can manifest in young and healthy patients, but well-known risk groups are pregnant women/neonates, the elderly, and immunocompromised people (New York State Health Department, 2024).

Listeria monocytogenes is a highly adaptable pathogen capable of surviving under various adverse environmental conditions, including refrigeration temperatures, high acidity, salinity, and low water activity. For example, *Listeria monocytogenes* has shown resilience in ready-to-eat (RTE) foods, which often rely on refrigeration and have limited use of chemical preservatives. The bacteria can endure environmental stressors like acidic conditions and osmotic stress, making it challenging to control without thermal processing or alternative preservation methods like lactic acid bacteria (LAB) as a bio preservative strategy. This highlights the pathogen's ability to survive in diverse and unfavorable environments, supporting its characterization as a resilient microorganism capable of thriving under conditions that would inhibit other pathogens (Webb et al., 2022), it can also survive freezing temperatures of -18 °C for months in different foods (ELIKA, 2023). This pathogen can be found everywhere in the environment and has been identified in water, soil, dust, plants, animal feeds, feces, and sewage (Webb et al., 2022).

This pathogen can form biofilms, which are defined as complex three-dimensional structures, irreversibly attached to a biotic or abiotic surface, and embedded in a matrix chiefly composed of extracellular polymeric substances (EPS) (Robinson et al., 2021). The ability of microorganisms to form biofilms is an adaptive and resistance strategy, which allows them to increase the availability of nutrients for their growth, facilitates the use of water, enables the transfer of genetic material, and

what is most worrying for the food industry, gives them resistance to antimicrobial agents (González-Rivas et al., 2018). Biofilms of *L. monocytogenes* on food contact surfaces have been identified as an important pathway for pathogenic persistence and subsequent product contamination (Huang, 2020). Moreover, according to Simões et al. (2009). The complex nature of biofilms and the capacity of the cells that make them up to strongly fix surfaces that are difficult to access make the action of the disinfectants currently being applied less effective.

Listeria monocytogenes can be introduced to dairy processing environments through dairy cows carrying the bacteria without clinical signs of the disease and can contaminate dairy products (Castro et al., 2018). On very rare occasions, mastitis in ruminants has been associated with an infection by *L. monocytogenes* (Almqvist et al., 2021). In the other hand, in the food industry, the formation of biofilms is favored by defective cleaning and disinfection, the presence of irregularities, pores, defects, or cavities on the surfaces of equipment and installations, as well as residual dirt, moisture, and nutrients. An inadequately cleaned food-processing equipment (e.g., stainless steel (SS) surfaces) constitutes a potential source for *L. monocytogenes*, resulting in contamination of food that meets such equipment. Even though adherence to strict sanitation practices should minimize the risk of the persistence of biofilms on surfaces after cleaning procedures, biofilms can remain due to their complex structure and ability to protect embedded bacteria from disinfectants. The biofilm matrix, primarily composed of extracellular polymeric substances (EPS), acts as a physical barrier that prevents the penetration of antimicrobial agents, making it difficult to effectively clean surfaces. A study published in 2022 discussed how the ECM provides a protective environment for microorganisms, contributing to the biofilm's resistance to antibiotics and disinfectants (J.-S. Kim et al., 2023) while certain microorganisms within biofilms can enter a dormant or "persister" state, which makes them highly tolerant to conventional treatments. These persister cells can survive antibiotic exposure due to their reduced metabolic activity, which is a common survival strategy within biofilms. Research from 2019 highlights that non-dividing or slow-growing cells in biofilms exhibit high tolerance to antimicrobial agents, further complicating eradication efforts. These biofilms in chronic

wounds can lead to repeated infections that are hard to manage due to the protective matrix that shelters pathogens from both the host immune response and antimicrobial treatments. This matrix also facilitates a reduced metabolic state in some bacteria, making them even more resistant to antibiotics and cleaning agents. Existing evidence suggests that a considerable risk may occur in sites of processing plants that are not easily cleaned or sanitized, such as those that do not allow direct access to sanitation equipment for abrasion (McClements & Conniff, 2023).

It has been shown that a combination of mechanical and chemical methods is necessary to effectively control biofouling, as chemical methods alone are often insufficient to completely eradicate the biofilm without mechanical disruption (Singh et al., 2023). Therefore, biofilm bacteria exhibit different properties compared to planktonic (free-floating) bacteria, which are often more susceptible to antimicrobial agents. The structural complexity of biofilms, combined with their ability to alter the efficacy of sanitizers through enzymatic inactivation and other mechanisms, makes them particularly resilient to cleaning efforts (Liu et al., 2023). This lack of efficient cleaning procedures can lead to microbial cross-contamination, standing as a critical concern within the industrial landscape, with its potential repercussions amplifying the gravity of its significance. The ramifications of a single tainted product extend beyond mere economic losses, as the slightest contact with other finished or processing items can cascade into the forfeiture of entire product batches (Møretrø & Langsrud, 2004).

Chemical agents and mechanical forces are parameters often involved simultaneously in the sanitation and removal of biofilms since applying sole chemical agents tends to leave the biofilm intact when no mechanical treatment is implemented in the control process (Bridier et al., 2015). Another factor to consider is pathogenic microorganisms' ability to resist current antimicrobial agents when cells form biofilms (Olsen, 2015). Therefore, attached cells are more difficult to mechanically remove from surfaces and are more resistant to sanitizers than their free-living counterparts (Liu, 2019). This lack of efficient cleaning procedures can lead to microbial cross-contamination, standing as a critical

concern within the industrial landscape, with its potential repercussions amplifying the gravity of its significance.

Attachment to surfaces is important for the survival and persistence of this pathogen in food processing environments, being the major concern is that it can easily adhere to all the materials commonly used in the food industry (Bridier et al., 2015). The biofilms formed in the dairy industry contain significant milk residues, particularly protein and minerals such as calcium phosphate creating a perfect environment for its formation. It's important to consider that one of the most important factors affecting biofilm structure and behavior is the velocity field of the fluid in contact with the microbial layer (Ulusoy & Chirkena, 2019). This effect is primarily due to the hydrodynamic forces exerted by the flowing fluid, which can affect how nutrients and oxygen are delivered to the biofilm and how waste products are removed because a higher fluid velocity can enhance the diffusion of nutrients and oxygen into the biofilm, promoting microbial growth and biofilm expansion. Conversely, lower velocities may limit nutrient diffusion, creating gradients within the biofilm that can lead to heterogeneity in microbial activity and structure (Junpeng Zhang et al., 2019).

Surface roughness increases the surface area available for bacterial attachment and provides a scaffold for adhesion (Stuermer, 2023). Food contact surfaces like stainless steel (SS) have low surface roughness for easy cleaning. Stainless steel is made primarily from iron and carbon in a two-step process. What makes stainless steel different is the addition of chromium (Cr) and other alloying elements such as nickel (Ni) to create a corrosion-resistant product (Burnett, 2023). The property of these alloys that makes them desirable, and which gives these alloys the name "stainless," is a very slow corrosion rate in most ordinary atmospheres such as elevated temperatures (Junpeng Zhang et al., 2019).

Other industrial surfaces such as floors and sinks have been highlighted as potential focuses of pathogen biofilm formation and as an initial route point for *L. monocytogenes* transfer to other surfaces. It has been demonstrated that this foodborne pathogen can survive for long periods on industrial surfaces and can be transferred to food products, thus compromising its innocuity

(Manufacturing.net, 2016). Floors of dairies and other premises used for processing milk have been problematic for many years and are the subject of many inquiries in the industry. The problem is choosing a floor capable of withstanding heavy impact and resistance to mildly corrosive liquids. The floor of a dairy should have a surface that is easily cleaned, and which is not slippery even when wet. Ceramic tiles are known for their durability and can withstand heavy foot traffic, equipment movement, and the occasional impact from dropped tools or equipment (Tina Mazaheri et al., 2021). In a laboratory view, mechanical removal of *Listeria monocytogenes* using glass beads involves physical agitation to dislodge bacterial cells from surfaces (U. Kim & Oh, 2024). This method is effective due to the abrasive action of the glass beads, which scrape off the bacteria. Studies show this technique significantly reduces bacterial adhesion on both stainless steel and ceramic surfaces (Skowron et al., 2019). In the food processing sector, it is common practice to first use mechanical cleaning techniques such as scrubbing, brushing, and high-pressure washes to remove as much of the biofilm as possible before applying chemical treatments. This combination helps ensure that the biofilm is adequately broken down and that microorganisms are not shielded by the biofilm matrix, leading to more effective sanitation outcomes (Bampidis et al., 2020). Combined with chemical cleaning agents, the effectiveness of bacterial removal increases, providing a comprehensive approach to maintaining hygiene in food processing environments (Fagerlund et al., 2020). These strategies integrate mechanical, chemical, and sometimes enzymatic treatments to enhance the effectiveness of biofilm eradication. Combining methods can target different components of the biofilm, such as the extracellular matrix and the microbial cells, leading to more thorough cleaning (Bridier et al., 2011).

This project aimed to evaluate the efficacy of biofilm detachment through mechanical action on stainless steel and ceramic surfaces inoculated with *Listeria monocytogenes* in whole milk and refrigerated at 4 °C for 10 days, and to determine how mechanical action reduces the percentage of contact spread of *Listeria monocytogenes* through microbial cross-contamination from stainless steel surfaces to fresh milk.

Materials and Methods

Experiment Location

The study was developed at the Animal and Food Science Laboratory at Louisiana State University in the city of Baton Rouge, Louisiana, United States.

Experimental Design

This experiment used an independent sample to compare a greater biofilm detachment between stainless steel and ceramic surfaces simulating mechanical force as a cleaning strategy through a t-test at 95% significance. The experiment included three repetitions, with four replicates each to ensure the robustness and reproducibility of the results. The treatment involved mechanical action using glass beads for two minutes to detach the biofilm membrane on each surface separately.

The second experiment evaluated the bio-transference of *Listeria monocytogenes* to food contact surfaces. The study was conducted using three different time intervals, with each interval comprising four repetitions and four replicates. Measurements were taken before applying mechanical force using glass beads and again after the application. This experimental design allowed for a comparative analysis of *L. monocytogenes* transfer across time points, providing insights into the effectiveness of mechanical force in reducing microbial contamination on food contact surfaces.

Inoculum Preparation

L. monocytogenes (V7, serotype ½ a, mil associated outbreak strain) was obtained from the Food Science & Microbiology laboratory at Louisiana State University, and it was used throughout the study.

The frozen cultures stored at -80 °C were thawed in a biological safety cabinet. The cultures were then activated by transferring 0.1 mL of 0.5 McFarland units to 10 mL of Tryptic Soy Broth (TSB) containing 0.6% yeast extract (TSBYE) and incubating at 37 °C for 24 hours. This activation process was repeated after 24 and 48 hours. After 48 hours of activation, the enriched cells were harvested by centrifugation at 7000 rpm for 15 minutes at 4 °C and washed with 1X Phosphate Buffer Saline (PBS).

The inoculum was prepared by the inoculum level was fixed to 10^8 CFU/mL, and then 10 mL of it was mixed with 90 mL of milk to be used as inoculum.

Surface Preparation, Inoculation, and Biofilm Formation

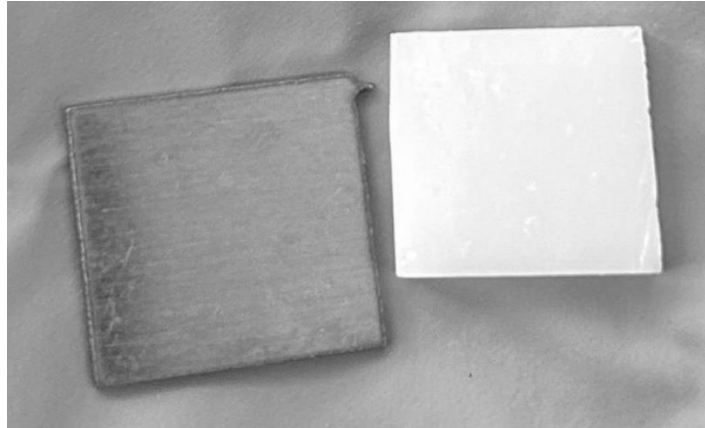
Coupons of stainless steel of 1 cm^2 (BioSurface Technologies Corp BST 5050304) and ceramic tiles coupons of 1 cm^2 (sweny) (Figure 1). The stainless-steel coupons were selected to mimic food contact surfaces like bulk milk storage tanks, pipelines, and milk pasteurizers. The ceramic tiles coupons were chosen to represent dairy processing facilities such as floors, walls, and drain areas.

Before initiating the biofilm study, the coupons were cleaned by submersion in alcohol at 70% for 30 min to 1h, then washed three times with distilled water to remove any alcohol residue. The clean coupons were airdried for 30 min to 1 hour and were sterilized with UV light for 15 minutes for each side. Each coupon was kept in each well of 12-well plates, immersed in 5 mL of inoculum (10^8 CFU/mL) in milk, and incubated at $37\text{ }^\circ\text{C}$ for 7-10 days to allow the bacteria to attach and form biofilm onto the surface.

To evaluate the formation of *Listeria monocytogenes* biofilms, 90 mL of whole cow's milk was used as a growth medium and inoculated with a concentration of 10^8 *Listeria*. The milk, rich in nutrients such as proteins, fats, and sugars, provided a favorable environment for bacterial growth. Additionally, the slightly acidic pH of milk (between 6.5 and 6.8) was ideal for the development of *Listeria*. Stainless steel and ceramic surfaces were submerged in the mixture of milk and *Listeria* to ensure that biofilm growth occurred on both surfaces. The samples were then refrigerated for 10 days at $4\text{ }^\circ\text{C}$ to simulate the storage conditions of finished products and raw materials in a processing plant. Although the low temperature limited active growth, the high relative humidity in refrigeration favored bacterial adherence and biofilm formation. The stainless steel, with its lower porosity, and the ceramic, more porous, exhibited different characteristics in terms of bacterial retention and detachment, demonstrating how each surface could influence the persistence of biofilms under typical industrial conditions.

Figure 1

Stainless steel (left) and ceramic tile (right) coupon.



Extraction of the Bacteria from Biofilm

To simulate the mechanical force, previously sterilized glass beads (10) were introduced into test tubes containing 10 mL of 1X PBS (phosphate buffer saline), a universal diluent, which already had the coupons inside, then vortexed for 2 minutes each. This procedure was performed in triplicate to ensure experimental reproducibility. The glass beads facilitated the thorough mixing and suspension of bacteria, promoting uniform conditions across samples (Konrat et al., 2016). The same extraction process was replicated for the ceramic tile coupons.

Then it was serially diluted and spread plated onto TSA with 0.6% YE incubated at 37 °C for 48 hours and enumerated. This process was duplicated to ensure the reliability of the results and facilitate a comprehensive analysis of bacterial concentrations at varying dilution levels.

Following this, the ceramic tile samples underwent a thorough cleaning process, which involved three washes with phosphate-buffered saline (PBS) and two minutes of agitation to ensure even distribution. After cleaning, serial dilutions ranging from 10^{-1} to 10^{-6} were prepared. Each dilution was labeled, and 100 μ L aliquots from the 10^{-3} dilutions were spread-plated. The samples were then incubated at 37 °C for 48 hours.

Bio Transfer

The stainless-steel coupons were inoculated with *Listeria* ten days prior, the stainless-steel samples were placed in a pre-sterilized beaker and submerged in 5 mL of fresh milk for three different time intervals (1, 2, and 5 minutes). To simulate the bio-transference of produce with *L. monocytogenes*. According to a study by Fong et al. (2014), the transfer of bacteria from stainless steel surfaces to a liquid such as water or milk can range from 0.1% to 2% within 1 minute, depending on the initial bacterial concentration and experimental conditions.

After each specified time interval, 1 mL of the milk was carefully extracted and subjected to a serial dilution up to a 10^{-1} concentration. This diluted sample was then spread plate into TSA. 0.6% YE. The process was repeated three times, with each repetition involving four replicates to ensure statistical accuracy and reproducibility.

The samples were subsequently incubated at 37 °C for 48 hours to allow for bacterial growth. Following the incubation period, colony-forming units (CFUs) were counted to quantify the bacterial transfer. This systematic approach allowed for a detailed assessment of the bacterial transfer kinetics from stainless steel to milk, providing insights into contamination risks during short-term exposure.

Results and Discussion

Extraction of the Bacteria from Biofilm

The data suggests a significant difference between the mean values of the two surfaces (ceramic tiles and stainless steel) (Table 1). The stainless-steel surface has a considerably higher mean value at 7.7433 Log CFU/mL. This suggests that, on average, the measurements of the detachment of *Listeria* from this surface through mechanical action are greater than detachment from the ceramic tile surface demonstrated what was mentioned by Junwen Zhang et al. (2021) “surface roughness increases the surface area available for bacterial attachment and provides a scaffold for adhesion”. Also, stainless steel (SS) shows less variability than the ceramic tile surface. This indicates that the values for the ceramic tiles (CT) surface are more spread out around the mean compared to the stainless-steel surface, this means that the detachment of *Listeria* from the ceramic tile surface is inconsistent and may be associated with its high porosity. The p-value (<0.0044) confirms that this difference is statistically significant. The standard deviation for stainless steel (0.1965) suggests that the measurements are more consistent and closer to the mean and are not due to random variation than the SD for ceramic tiles (0.9587).

The standard error for ceramic tiles (0.2768) is larger than that for stainless steel (0.0565), indicating greater variability in the mean estimate for ceramic tiles compared to stainless steel. Statistically, a smaller Standard Error suggests a more precise estimate of the true population mean, thus, the mean microbial detachment of 7.7433 log CFU/mL for stainless steel is more reliable and closer to the true mean than the 6.7525 log CFU/mL for ceramic tiles. This means that stainless steel surfaces are generally smoother and less porous than ceramic tiles, leading to more consistent microbial detachment measurements due to uniform adhesion and detachment processes. In contrast, the rougher and more porous texture of ceramic tiles can cause greater variability in microbial adhesion, contributing to the higher SE observed. Recent research supports this: (Rodriguez et al., 2008) found that increased surface roughness and porosity are associated with higher microbial attachment and biofilm formation, leading to greater variability in detachment measurements.

Similarly, U. Kim and Oh (2024) highlighted that surface characteristics, such as roughness and texture, significantly impact microbial adhesion and detachment, further explaining the observed differences in precision between stainless steel and ceramic tiles.

Table 1

Bacterial detachment from food and non-food contact surfaces.

Surface	N	Log CFU/mL of milk \pm SD	Std Err
Ceramic Tiles	12	6.7525 \pm 0.9587	0.2768
Stainless Steel	12	7.7433 \pm 0.1965	0.0565
P <0.0044			

Bio Transference

The transfer of bacteria from previously treated stainless steel (SS) surfaces to uninoculated fresh milk (Table 2) increased as exposure time lengthened. For samples where mechanical force was applied, the bacterial transfer showed a significant reduction at both 1 minute and 2 minutes, with probability values of less than 0.0001, indicating that there was no significant bio transference of *L. monocytogenes* from the stainless-steel surface to the milk within these short contact times. At 5 minutes, the probability value was 0.0001, showing a significant reduction in bacterial spread, although less pronounced compared to the shorter time points, with the spread measurement decreasing from 3.83 to 2.64. This suggests that while bacterial transference occurs, it is less predictable over longer periods. In contrast, for samples that did not use mechanical force, bacterial transfer typically showed less pronounced reductions and greater variability. Studies have shown that mechanical force can enhance the detachment of biofilms and reduce bacterial transfer more effectively compared to passive contact, as mechanical agitation disrupts bacterial adhesion and biofilm integrity (Zhao et al., 2021). This underscores the effectiveness of mechanical force in controlling bacterial transfer and emphasizes the variability in bacterial spread over time in the absence of such force.

Mechanical force disrupts and partially removes the biofilm, leading to a smaller and less robust biofilm, which results in reduced bacterial transfer that may fall below the detection limits of

quantification techniques (Simões et al., 2005). As contact time increases, however, there is more opportunity for bacterial transfer to the milk, resulting in measurable quantification of bacterial presence. This is because extended exposure allows for more bacteria to migrate from the weakened biofilm to the milk, providing quantification that surpasses that from an undisturbed biofilm (Vishwa, 2019). Thus, while mechanical action initially reduces bacterial transfer, longer exposure times enable better detection and quantification of bacterial contamination. Minimizing contact time between pre-treated surfaces and fresh foods is crucial to reducing the risk of cross-contamination. Research shows that shorter contact times between contaminated surfaces and food significantly lower the likelihood of microbial transfer, thereby reducing the risk of foodborne illnesses. For instance, Kaylegian and Rolon (2023) found that decreasing the contact time between contaminated surfaces and food effectively lowers microbial transfer rates and helps mitigate the risk of cross-contamination. Similarly, U. Kim and Oh (2024) demonstrated that reducing contact time between surfaces and food products led to a significant decrease in microbial transfer, with statistical analyses confirming the reliability of these findings.

Table 2

Bacterial transference before and after the application of mechanical action.

<i>Surface</i>	<i>Bio transference time</i>	<i>Growth Before Media ± SD Log CFU/mL of milk</i>	<i>Growth After Media ± SD Log CFU/mL of milk</i>	<i>Probability</i>
Stainless Steel (SS)	1 minute	2.90 ± 0.15	0 ± 0	<.0001
	2 minutes	3.44 ± 0.13	0 ± 0	<.0001
	5 minutes	3.83 ± 0.14	2.64 ± 0.94	<.0001

Discussion

The project aimed to understand *Listeria monocytogenes* detachment from two different surfaces (ceramic tiles and stainless steel) under mechanical stress and its implications for food safety in the dairy industry. The focus was on comparing ceramic tiles (CT) and stainless steel (SS) surfaces to determine which posed a higher risk of bacterial contamination. The findings revealed

that the stainless-steel surface, with a higher mean detachment value of 7.7433, exhibited greater bacterial detachment compared to ceramic tiles. This is significant for food processing facilities that use stainless steel due to its durability and ease of cleaning, as noted by (Sehgal et al., 2024), who emphasize its non-reactive and stable properties. However, biofilms can accelerate the corrosion of stainless steel, making effective cleaning protocols crucial (Ghelichkhah et al., 2021). Stainless steel surfaces generally facilitate easier biofilm removal, but they also require rigorous cleaning due to their potential for microbial contamination (Tallawi et al., 2017). Ceramic tiles, due to their roughness, are more challenging for biofilm detachment, leading to higher bacterial retention (Pogreba-Brown et al., 2021).

Minimizing contact time between contaminated surfaces and fresh milk is essential for reducing cross-contamination risk. Short contact times at 1 and 2 minutes showed no bacterial transfer from stainless steel to milk, indicating that brief contact effectively minimizes contamination risk. At 5 minutes, there was an increase in bacterial transfer, highlighting the importance of reducing prolonged contact times for food safety, as noted by T. Mazaheri et al. (2023), who reported a potential global cross-contamination rate of 20.4% with untreated biofilms. The mechanical force was effective in reducing bacterial transfer in the initial 2 minutes, as Silva et al. (2020) found that physical agitation disrupts biofilm structure and enhances antimicrobial treatment effectiveness. However, variability at 5 minutes suggests that while mechanical force helps, it may not be consistently reliable over longer periods. The study underscores the need for stringent cleaning protocols, particularly for stainless steel surfaces, and emphasizes minimizing contact time to reduce cross-contamination risk. These insights highlight the critical role of surface properties, contact duration, and mechanical interventions in maintaining food safety in the dairy industry, guiding best practices for hygiene and contamination control.

Conclusions

Mechanical action effectively detaches *Listeria monocytogenes* biofilm from stainless steel surfaces, due to their smoother texture, facilitating biofilm disruption. However, mechanical action alone is insufficient for ceramic tiles, whose rougher surface enhances bacterial adhesion and complicates biofilm removal. This highlights the need for combining mechanical and chemical cleaning strategies to manage biofilms on various surfaces and ensure food safety effectively.

Mechanical action effectively minimizes *Listeria monocytogenes* biofilm transfer from stainless steel surfaces to fresh milk, with short contact times (1-2 minutes) resulting in undetectable bacterial transfer. However, longer contact times (5 minutes) led to increased biofilm transfer, highlighting that while mechanical intervention significantly disrupts biofilm integrity, the risk of cross-contamination rises with extended contact durations. This finding underscores the importance of not only employing mechanical cleaning strategies but also minimizing contact time between treated surfaces and food products to ensure effective prevention of biofilm-related cross-contamination.

Recommendations

Consider varying the duration of mechanical treatment using glass beads or exploring alternative mechanical action simulations to assess whether extended exposure time improves the detachment efficiency of *Listeria* from surfaces.

Use a combination of cleaning protocols that involve different concentrations of cleaning agents, like alkaline and acidic solutions, to weaken the membrane of bacteria. This allows chlorine to kill the bacteria effectively. Pair this with the mechanical action of glass beads to help remove any remaining bacteria attached to the surfaces.

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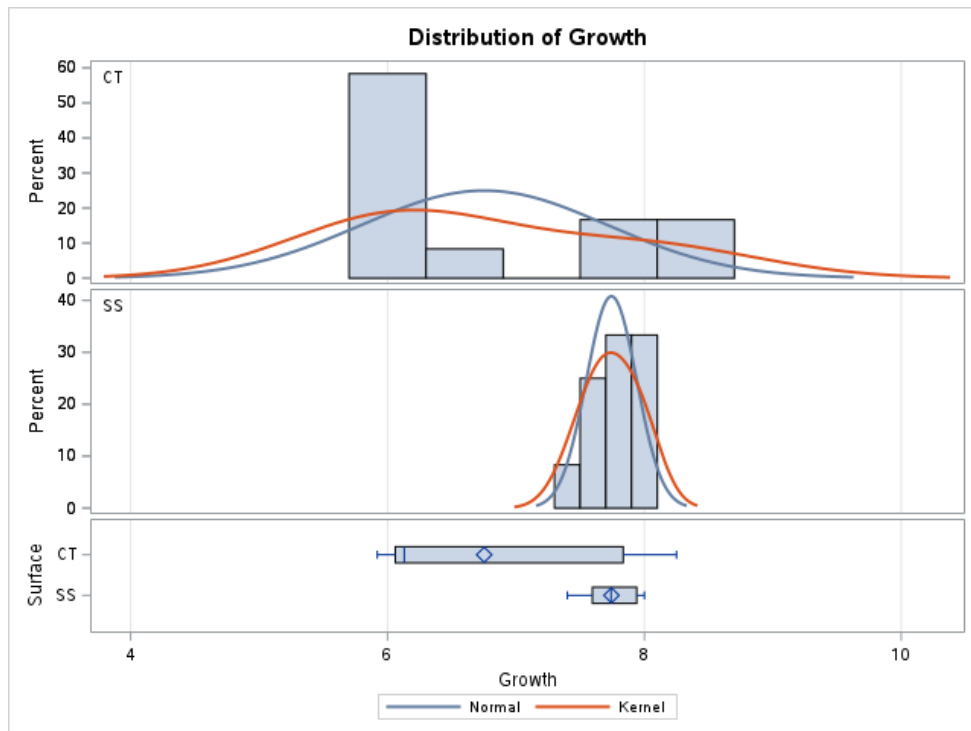
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Appendices

Appendix A

Growth Behavior Curve



Appendix B*Glass Beads*