Control of *Listeria monocytogenes* in Cheese Brines: A Literature Review

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ABSTRACT

Listeria monocytogenes (*L. mono*.) is a top pathogen of concern in the cheese industry. The organism is prevalent in the environment and has been known to inhabit dairy manufacturing facilities, including long-term survival in brine tanks. Along with good hygiene and sanitation practices, validated *L. mono*. control methods must be employed to ensure extended-use brine tanks do not become reservoirs for *Listeria monocytogenes*, potentially leading to serious health issues in cheese consumers. Control methods considerations include *L. mono*. cells in suspension, cells attached to protective biofilms, and effectiveness in the presence of organic matter. This literature review investigated the availability of scientific research on effective control methods for *Listeria monocytogenes* in cheese brines. Research on *L. mono*. control in other applicable areas was considered where information related specifically to cheese brine environments was not found. Potential effective control methods were identified, including membrane filtration and oxidizing agents such as peroxyacetic acid and hydrogen peroxide. Further research is needed to fill knowledge gaps related to successful implementation of these controls in cheese brines.

Contents

Introduction

Background and Setting

Listeria monocytogenes (*L. mono*.) is a pathogen of concern in the dairy manufacturing industry. This organism is common in the environment and can be inadvertently transported into food manufacturing facilities on the shoes and clothing of people, on incoming ingredients and materials, and transported around the facility by fork trucks and other traffic. Once inside a facility, *L. mono*. often finds harborage sites or growth niches that are difficult to clean and provide ideal growth conditions (Ferreira, 2013). *L. mono*. has been known to survive in these conditions for years or even decades (Carpentier & Cerf, 2011).

Cheese manufacturing facilities present a particularly attractive environment for *Listeria monocytogenes* due to the high levels of moisture and cool temperatures of these environments. Sampling studies of cheese-manufacturing facilities have found persistent, wide-spread presence of *Listeria monocytogenes* despite elaborate cleaning and sanitation programs (Stessl et al., 2014), including a serotype associated with listeriosis outbreaks found in a brine solution (Barancelli et al., 2011).

Cheese brine tanks are occasionally considered to be safe from microbiological contamination due to the high salt content, but research has consistently disputed this assumption. Research articles have documented the presence of *Listeria monocytogenes* in cheese brine samples taken from cheese manufacturing facilities (Barancelli et al., 2013; Wagner, Eliskases-Lechner, Rieck, Hein, & Allerberger, 2006). Larson, Johnson, and Nelson (1999) conducted laboratory studies on the survival of *L. mono*. in commercial cheese brines. They inoculated 38 brine samples from a variety of cheese types with a mixture of *L. mono.*

strains and noted survival for over 200 days in many of these inoculated samples, with sodium chloride levels ranging from 16.9% to 23.8%. A similar study conducted by Boyer et al. (2009) found no significant *L. mono*. population reduction in chill brines with salt levels of 7.9% and 13.2% incubated at 4^{o}C and 12^{o}C for ten days.

A characteristic of controlling *Listeria monocytogenes* is the formation of biofilms in its environment. Biofilms are communities of cells embedded in a polymer matrix and irreversibly attached to a surface, which exhibit altered growth rate and gene transcription as compared to non-attached cells (Donlan and Costerton, 2002). These biofilms provide protection from antibacterial agents. High salinity conditions of greater than 11% sodium chloride have been shown to decrease the adhesion capability of Listeria monocytogenes (Caly, Takilt, Lebret, & Tresse, 2009), so biofilms are not likely to form in these conditions. Research conducted on biofilm control is included in this study because it addresses the control method's ability to inactivate *Listeria monocytogenes* even in this protective state.

Listeria monocytogenes is known to be a frequent resident in cheese manufacturing facilities. If the organism is not properly controlled in the environment and makes its way onto food contact surfaces, it can contaminate these ready-to-eat products and cause serious illness to consumers.

Statement of the Problem

Cheese brines are often used for extended periods of time due to the expense and environmental concerns related to disposing high salt solutions and to minimize lost production time associated with emptying the brine tank to conduct a thorough cleaning. Contamination from environmental sources can persist in brine and has the potential to lead to foodborne illness outbreaks. No regulations dictate length of time cheese brines may be used or prescribe approved methods to control pathogenic bacteria.

Regulating agencies recognize the importance of controlling brine systems for *L. mono.*, but do not specify appropriate methods or processes. Codex Alimentarious Guidelines state that reused brine that directly contacts finished product should be decontaminated via chlorination, heat treatment, or “some other effective treatment” (Codex, 2007). Likewise, the Food and Drug Administration’s Draft Guidance on the Control of *Listeria monocytogenes* in Ready-to-Eat Foods recommends using measures such as chlorination, ozonation, heat treatment, or “other effective treatment” to control this pathogen (FDA, 2017) but leaves the interpretation and implementation of this guidance up to individual food manufacturers’ discretion.

Although the importance of controlling *L. mono*. in cheese brines is understood, there is generally a lack of consensus and readily available research on the best methods to provide adequate control in this environment.

Significance of the Problem

In most naturally produced cheese processes, there are no validated kill-steps in place to control pathogens after the initial pasteurization of the milk. Environmental contamination by *Listeria monocytogenes* that occurs later in the manufacturing process, such as during brining, may persist in the finished product. Most cheeses are considered ready-to-eat products, with no cooking step required prior to consumption.

Exposure to *Listeria monocytogenes* can have dire consequences, particularly if consumed by vulnerable populations. While other bacterial agents such as *Salmonella* and *Campylobacter* have more frequent outbreak occurrences, *L. mono* is particularly concerning due to the virulent nature of the organism. Listeriosis infection symptoms can be quite severe and may include septicaemia (blood infection), meningitis, encephalitis, corneal ulcer, pneumonia, and spontaneous abortion or stillbirth in pregnant women (Griffiths & Walkling-Ribeiro, 2012). In 2012 *L. mono.* was ranked seventh on the Center for Disease Control's list of bacteria-related foodborne illness outbreaks, but it was the top bacterial cause of foodborne illness deaths (CDC, 2014).

Validated control methods are needed to ensure food safety and limit the scope of contamination events.

Purpose of the Project

The purpose of this project is to investigate methods to effectively and efficiently control *Listeria monocytogenes* in cheese brines, as identified through a review of scientific research studies. This includes the investigation of control methods researched regarding other food manufacturing environments where lack of research exists for cheese brines. The ready-to-eat meat industry also considers *L. mono*. a pathogen of concern and utilizes chill brines that are similar in composition to those used in the cheese industry. Research in this area may be particularly applicable to this project.

Project Objectives

The objectives of this project are to summarize available research on the control of *Listeria monocytogenes* in cheese brines or related applications, identify gaps in knowledge, and provide recommendations for future research needs in this area.

Review of Literature

Acidification

A fundamental strategy in food preservation is to lower the pH of the environment to make it inhospitable to microbial growth. This method has been used successfully in many applications for years including pickling and canning. This strategy has been investigated for its usefulness in controlling pathogens in cheese brines. Acidification of brine using food grade acetic acid (AA), lactic acid (LA), hydrochloric acid (HCl) and citric acid (CA) to control *Listeria monocytogenes* in cheese brines was investigated by Brown, Millan-Borrero, Carbonella, Micheletti, and D'Amico in 2017. This study inoculated prepared brine with the addition of 2% whey with *L. mono*. These researchers found that the *L. mono*. survived in untreated brine at >5 log CFU/mL for 50 days when stored at 12^{o}C. Treatment with acids was successful in obtaining a 5-log reduction in varying amounts of treatment times:90 minutes with LA and AA, 105 minutes with CA, and 135 minutes with HCl.

Biofilms of *L. mono*. attached to PVC or stainless steel surfaces were not inactivated through exposure to lactic acid alone in one study (Ban, Park, Ryu, & Kang, 2012), while another found a lactic-acid based sanitizer to be effective against *L. mono*. biofilms on a high density polyethylene cutting board (Yang, Kendall, Medeiros, & Sofos, 2009). Biofilm control with lactic acid may be dependent on the material to which the biofilm is attached.

While acidification may represent a simple, low cost solution to controlling microbial populations in certain situations, researchers have documented the ability of *L. mono*. to develop a tolerance to acidic conditions. Lou and Yousef (1997) examined *Listeria monocytogenes* populations that were subjected to sub-lethal exposure to acidic conditions. These ‘stress hardened' cells subsequently became highly resistant to extremely acidic conditions. Similar research found that prior exposure to low pH resulted in a significant increase in resistance to pH 3.5 in multiple strains of *Listeria monocytogenes* (Skandamis, Yoon, Stopforth, Kendall, & Sofos, 2008). The level of adaptive response is dependent on other environmental factors, including additional stressors such as temperature or salt (Koutsoumanis, Kendall, & Sofos, 2003).

Acidification has proven to be effective in controlling *L. mono*. in unattached cells in brine but must be used with consideration of the organism's ability to adapt to an acidic environment.

Oxidation

Oxidizing agents are frequently considered control methods for *Listeria monocytogenes* in food manufacturing. There are many commercial formulas employed by chemical companies that cater to the industry. All of these oxidizing agents cause the target organism to lose an electron, disrupting important cellular structures. Often this will result in disruptions in the cell wall, which can cause components to leak out of the cell, or impacts on important structures inside of the cell, like enzymes or DNA. Following is a review of a few well-studied oxidizing chemicals used in the food industry to control *Listeria monocytogenes*.

Chlorine Dioxide

Chlorine dioxide (ClO_2) is an oxidizing agent that was investigated as a disinfectant to replace chlorine in routine water treatment when it was discovered that chlorine disinfection led to the creation of harmful disinfection by-products. It is a strong disinfectant that kills bacteria and inactivates viruses within a wide pH range, requires a smaller dose and faster reaction time than chlorine (Chang, Hsieh, Shih, Hsu, & Wang, 2000). Studies with mice concluded that a purified form of chlorine dioxide was safe to use and did not cause eye irritation at 50 ppm, inhalation irritation at 20 ppm, or oral toxicity at 40 ppm (Ma et al., 2017).

No published studies were found that examined the use of chlorine dioxide for controlling *L. mono*. in the dairy industry, but other work has been done that may be applicable. Valderrama, Mills, and Cutter (2009) examined the use of ClO_2 in chill brines in the ready-to-eat meat industry. In fresh brine solutions with a population of approximately 7 log cfu/mL of *L. mono*., 3 ppm of ClO_2 resulted in a 4-log cfu/mL reduction of the pathogen within 90 seconds of treatment. In used brines, however, the same treatment resulted in no significant reduction in *L. mono*. population. This was attributed to the organic material present in the spent brine depleting the ClO_2 so that it became unavailable to inactivate the *L. mono*. The researchers suggested the removal of organic matter prior to using chlorine dioxide to control *L. mono*. in spent brines.

Trinetta, Vaid, Xu, Linton, and Morgan (2012) studied the impact of chlorine dioxide gas on *L. mono*. biofilms on stainless steel surfaces. They found a greater than 3 log cfu/cm^2 reduction in *L. mono*. using a relatively low concentration (0.3 mg/l) of chlorine dioxide in a 10-minute application. A higher concentration (2 mg/l) and longer exposure time (30 minutes) were required to completely eliminate *L. mono*. in their experiments.

Chlorine dioxide is effective in reducing *Listeria monocytogenes* counts in unattached cells in brine and on biofilms attached to stainless steel surfaces and has been shown to be safe when used in a purified form. A potential drawback to this solution is reduced efficacy in the presence of organic matter.

Ozone

Ozone is a strong oxidizing agent that kills bacteria by destroying their cell walls. It can be used by dissolving into water (aqueous) or in a gaseous form. Varga and Szigeti (2016) summarized the use of ozone in the dairy industry and highlighted the usefulness of ozone treatments in reducing bacterial counts in several areas of dairy production including the indoor atmosphere of cheese ripening facilities and in reducing milk residue and biofilms on stainless steel surfaces.

Marino et al. (2015) studied the use of gaseous ozone in used dairy brines. Their investigation involved six used dairy brines with salt contents ranging from 11.25% to 16.96%. A pre-ozone test found no *L. mono*. or other pathogens present, but populations of other microorganisms from 4.7 log cfu/mL to 6.43 log cfu/mL. The brines were treated with gaseous ozone and obtained a greater than 3 log cfu/mL reduction in micro counts in 240 minutes. Higher protein levels in brine negatively impacted the effectiveness of the treatment and would likely require longer contact time to reach the desired effect. Although the study did not specifically address *L. mono*., the authors point to this technology as a potentially inexpensive but effective control method for microorganisms in brines.

A similar study in apple juice obtained positive results with foodborne pathogenic organisms. Researchers Choi et al. (2012) investigated the time to achieve a 5-log reduction of foodborne pathogens using ozone. *E. coli.* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* were the target organisms in this study. Ozone treatment was effective in inactivating the pathogens (including *L. mono*), but the efficacy was significantly impacted by the solids content of the apple juice.

Regarding biofilms, researchers Robbins, Fisher, Moltz, and Martin (2005) examined ozone’s effectiveness in eliminating *L. mono*. biofilms on stainless steel surfaces. These researchers found the planktonic (unattached) cells were completely destroyed by ozone.

Attached cells were also destroyed but required up to a 16-fold increase in ozone concentration versus the unattached cells.

Ozone is a strong oxidizing agent but its effectiveness may be negatively impacted by high protein levels that are often present in cheese brines. High concentrations and/or long contact times may be drawbacks to utilizing this control method.

Peroxyacetic Acid and Hydrogen Peroxide

The oxidizing agents peroxyacetic acid (PAA) and hydrogen peroxide (H_2O_2) have been studied for their effectiveness at inactivating *L. mono*. both alone and in combination with other sanitizing agents.

Lee et al. (2017) studied peroxyacetic acid on *Listeria monocytogenes* biofilms isolated from a cheese plant. They found this agent was effective on stainless steel surfaces, but only achieved a 1.8-2.5 log cfu/g reduction on polystyrene surfaces. Belessi, Gounadaki, Psomas, and Skandamis (2010) confirmed peroxyacetic acid's effectiveness on biofilms on stainless steel under food manufacturing conditions. Fatemi and Frank (1999) examined the effectiveness of sanitizers on biofilms in the presence of organic matter. They found that peroxyacetic acid combined with hydrogen peroxide and acetic acid (POA) was the most effective in inactivating mixed-culture biofilms of *Listeria monocytogenes* when milk was applied to the biofilm or introduced with the sanitizer treatment.

Robbins et al. (2005) concluded that hydrogen peroxide was very effective at eliminating *L. mono*. biofilms attached to stainless steel. Unattached cells were completely eliminated after exposure to 3% H_2O_2 for 10 minutes, while cells attached to biofilms were eliminated by 5% H_2O_2 exposure for 15 minutes. In this study, the effectiveness of this sanitizer was not impacted by the presence of high organic loads.

Brinez, Roig-Sagues, Herrero, Lopez-Pedemonte, and Guamis (2005) evaluated the effectiveness of peroxyacetic acid combined with hydrogen peroxide (PAHP) in the presence of organic matter. This study utilized milk, orange juice, liquid egg, and chocolate milk shake as interfering substances containing organic matter to mimic the conditions of a food manufacturing facility. All treatments resulted in a greater than 5 log cfu/mL reduction of *Listeria monocytogenes* in 10 minutes.

To summarize, oxidizing chemicals alone and in combination have proven to be effective at controlling *Listeria monocytogenes* both unattached and attached to biofilms and are not negatively impacted by the presence of organic matter. The effectiveness on biofilms may vary based on the material to which the biofilm is attached. Because these are oxidizing agents, they have the potential to impart undesirable flavor characteristics in exposed cheese which could disqualify their use in some applications.

Ultraviolet Light

Ultraviolet light refers to light with wavelengths ranging from 10 to 400 nanometers (nm), which is shorter than visible light but longer than x-ray. The form that is used in the food industry is UV-C, which is light with wavelengths of around 100 to 280 nm that kills bacteria by destroying their DNA (Barba, Koubaa, Prado-Silva, Orlien, & de Souza Sant'Ana, 2017). UV technology is used for disinfection of water supplies, sterilizing surfaces, and controlling harmful bacteria. Although UV exposure can be hazardous to people, modern treatment systems are designed to limit exposure while in use.

No published studies were found that examined the use of UV light for controlling *L. mono*. in cheese brines, but research is available relating to the ready-to-eat meat industry that may be applicable. McKinney, Williams, Boardman, Eifert, and Sumner (2009) studied the dosage of UV light required to inactivate *Listeria monocytogenes* in ready-to-eat meat brines. They found that UV was effective in inactivating *L. mono*. in water and fresh brine, but reduced transmission led to decreased effectiveness in varying concentrations of spent brines.

Other researchers evaluated the effectiveness of UV light in combination with other antimicrobial agents. Parikh, Williams, Mallikarjunan, Eifert, and Marcy (2011a) conducted a study of *L. mono*. in chill brines used in the ready-to-eat meat industry. In this study, fresh brine solutions were inoculated with *L. mono*. and exposed to UV with and without an antimicrobial agent. They found that the most effective reductions were seen in citric acid combined with ultraviolet light in 45 minutes of circulation. Dimethyl dicarbonate with UV also yielded significant population reduction but required more contact time than citric acid.

Kumar, Williams, Sumner, and Eifert (2015) investigated a combination of ozone and UV treatments on fresh and spent brines. The combination resulted in a >9 log cfu/ml reduction of *L. mono*. within a 25-minute treatment time in a fresh brine solution. When treating spent frankfurter brines, the result was a 5 log cfu/ml reduction that required 60 minutes to achieve. The difference in results was attributed to organic matter and particulates that were present in the spent brine, which decreases the penetration of UV into the brine solution.

Although researchers found UV light to be effective in inactivating *L. mono*. biofilms on stainless steel surfaces (Kim, Park, & Ha, 2016; Tajik, Naghili, Ghasemmahdi, Moradi, & Badali, 2015), fresh produce and packaging materials (Montogomery & Banerjee, 2015), this treatment method may not be practical for use on biofilms present in brine tanks containing high solid solutions.

UV technology has been reported to effectively control *Listeria monocytogenes* but has consistently shown to be negatively impacted by the presence of solids in the solution. The protein levels present in recycled cheese brines may present an obstacle to adoption of this in the cheese industry for pathogen control in brines.

Membrane Filtration

Membrane filtration utilizes a thin layer of semi-permeable material that separates substances based on size when a driving force is applied across the membrane. Common filtration systems include microfiltration, ultrafiltration, nanofiltration, and reverse osmosis (Minn. 2009). Microfiltration and ultrafiltration are considered in this review due to their current use and availability of information on these systems in this application.

Microfiltration is loosely defined as membranes with pore diameter from 0.2 µm to 5 µm in diameter. These can be used to filter out relatively larger particles such as sand, silt, clay, along with some bacteria. According to Low and Donachie (1997), *Listeria monocytogenes* cell size ranges from 1 – 2 µm long and 0.5 µm in diameter. *Eight L. mono.* strains were tested for their ability to pass through membrane filters of various pore sizes. All strains were found to have the ability to infiltrate membranes with pore sizes of 0.2 µm and larger. One *L. mono* strain (EGD) was tested on smaller membrane and did not pass through a 0.1 µm filter membrane (Nakazawa, Hasegawa, Nakagawa, Terao, & Matsuyama, 2005). Microfiltration has been investigated as an alternative to milk pasteurization, and although it is very effective in removing bacteria and spores it cannot guarantee complete removal of pathogenic bacteria in this application (Fernandez, Blanco, & Rodriguez, 2013).

A microbial challenge study conducted for Pall Corporation evaluated the effectiveness of their microfiltration system in removing *Listeria monocytogenes* from brines. The results of this study reported a greater than ten log reduction in numbers of *L. mono*. in filtrate compared to the inoculated brine prior to filtration (Brendel-Thimmel, Schilz, Unkelbach, & Heusslein, 2011). The system is promoted to provide clarification and microbial reduction either through continuous filtration, in which 5% to 20% of total brine volume is filtered during operation, or via batch mode, in which an entire batch of brine could be filtered overnight.

Ultrafiltration membranes generally have pore sizes between 0.002 to 0.1 µm in diameter. Although this technology is known to be used for this purpose and is advertised as a reliable defense against microorganisms, no published review studies were found in relation to ultrafiltration to control pathogens in cheese brines.

In any membrane filtration system, proper maintenance must be conducted to minimize the risk of inadvertent contamination of filtrate. Many systems utilize reverse flow to dislodge particles that accumulate at the membrane surface during use. If these particles are not removed from the system, they will concentrate at the membrane surface and introduce risk for concentrated retentate to pass through in the event of an integrity breach, such as a rip or tear in the membrane. Buildup of solids on the membrane can also result in low flow through the system, reducing the rate of filtration.

Summary of Outcomes, Discussions, and Recommendations

Extensive literature searches were conducted on all of the potential control methods mentioned above. In some cases, it was difficult to find published research related to *Listeria monocytogenes* control in cheese brines. Ready-to-eat meat chill brine and laboratory studies provided insight into potential control methods, but as outlined below further research is needed to validate their usefulness in this specific dairy environment. The lack of readily available information on *Listeria monocytogenes* control in cheese brines highlights a significant gap in knowledge on this important topic. Future study in this area would help cheese manufacturers ensure this potential food safety hazard is properly mitigated.

Discussion of Pathogen Control Methods

A few of the control methods investigated (acidification, ultraviolet light, chlorine dioxide, ozone) do not appear to be ideal methods for controlling *L. mono*. in cheese brines based on the studies found. Acidification has been shown to effectively reduce levels of *L. mono*. in the presence of organic matter, but the pathogen may develop a tolerance to acidic conditions in some environments. From the research results found, ultraviolet light does not appear to be a viable control method in this application due to its inability to penetrate solutions with high solids or organic matter that naturally accompanies cheese in a brine solution. Oxidizing agents chlorine dioxide and ozone also have shown to have reduced effectiveness in solutions with elevated protein levels, so these do not appear to be the most appropriate control methods in for pathogens cheese brine.

Two control methods investigated (membrane filtration and oxidizing agents peroxyacetic acid and hydrogen peroxide) have the potential to be utilized as effective control methods in cheese brine. Peroxyacetic acid and hydrogen peroxide have been shown to be effective in reducing populations of *L. mono*. in dairy environments and in the presence of organic matter. A potential limitation of these agents could be negative flavor impacts due to their oxidation reactions. No research was found that investigated flavor impacts of oxidizing agents in cheese brines. Continuous membrane filtration is another potentially viable control method for *L. mono*.

in cheese brines, but more information is needed. The filtration research studies that were found were limited, focused mainly on microfiltration, and were published by the filtration system manufacturers. Impartial evaluations of these methods are needed to confirm filtration's usefulness in this application.

Recommendations for Future Research

Future research needs have been identified that could provide extremely beneficial information on proper control of *Listeria monocytogenes* in cheese manufacturing facilities.

Membrane filtration of recycled cheese brines appears to be a viable solution, but limited subjective data is currently available. Validation studies for both microfiltration and ultrafiltration in spent cheese brines of varying salt concentrations and organic matter levels would be particularly useful for the industry.

Oxidizing chemical agents have shown to be effective in controlling *Listeria monocytogenes* in the presence of organic matter, but studies evaluating flavor impacts were not found. Investigations of peroxyacetic acid and hydrogen peroxide in controlling *L. mono*. in cheese brines along with an investigation of organoleptic impacts of these agents on cheese in brine would be of particular interest.

Combined control methods, in concert with good environmental control programs and upstream processes, may prove to be the most effective strategy to minimize the presence of *L. mono*. in cheese brines.

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