The Effect of Cheese Brine Concentrations on Survival of *Listeria monocytogenes*

Hisamettin Durmaz¹*, Osman Aygun², Mustafa Ardic¹

¹Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Harran University, Sanliurfa, Turkey
²Programme of Food, Suleyman Demirel Keban Vocational College, ²Firat University, Elazig, Turkey

Abstract: In this study, inactivation of *Listeria monocytogenes* at different salt concentrations and times were studied to gain a better understanding of the response of the bacterium in the brines. Cheese brines containing 13%, 15% and 19% NaCl were inoculated with 10⁴ *L. monocytogenes* (serotype 4b, RSKK 475) CFU/mL and stored at 4 °C for 90 days. Population of the pathogen in 13% brine decreased significantly (P<0.05) during first 30 days of storage compared to population in initial brine. However, *L. monocytogenes* was able to survive in 13% brine during 90 days of storage. Whereas the population of *L. monocytogenes* in the 15% brine decreased significantly between days 0 to 15 of storage so that direct planting at 30, 60 and 90 days gave negative results, but the same samples gave positive results after enrichment. Numbers of *L. monocytogenes* in the 19% brine decreased faster than mentioned above for other salt concentration and the pathogen was not detected in brine after 15 days of storage by both the direct planting and enrichment. As a result, it was suggested that brined cheeses should be stored in brines containing 19% or more NaCl for at least 15 days to prevent survival of *L. monocytogenes*. Keywords:

Key words: *Listeria monocytogenes*, brine, salt concentration

Introduction

The salting process is an important step in the manufacture of most cheese varieties. The salt in cheese has different functions, such as reduction of curd moisture, suppression of unwanted microorganisms, modification of flavour and texture and contribution to cheese ripening (Ibáñez et al. 1993; Mulvihill and McCarthy 1994; Laborda and Rubiolo 1999; Mulet et al. 1999). Therefore, in cheesemaking processes of some traditional cheese varieties, a high salt content in brine is essential for controlling microflora, preventing growth of pathogens and controlling enzyme activities during storage (Abd El-Salam et al. 1993).

Although storage in brine is thought to cause a decrease in the populations of undesirable contaminants, there is great concern that the brine can also serve as a reservoir of certain salt-tolerant pathogens. There have been a number of cases where especially brined cheeses have acted as carriers of foodborne infections (Keceli and Robinson 1997), because poorly maintained brine tanks can become ready sources of contamination. Since *Listeria monocytogenes* can survive after the pasteurization process (Doyle et al. 1987), they can easily be carried into commercial cheese brines. Because commercial cheese brines are mostly used repeatedly, the proteins and other nutrients from cheese are accumulated in brines, which make the brine a nutrient-rich environment. Larson et al. (1999) reported that *L. monocytogenes* survived for 118 days in fresh feta cheese brines (65 g/L NaCl and pH 6.8) at 4 °C and 12 °C; moreover, it has been shown that *L. monocytogenes* can grow in salt solutions of up to 60 g/L NaCl. Papageorgiou and Marth (1989) studied the fate of in salted whey. They found that the pathogen was able to grow in salted whey (60 g/L NaCl, pH 5.65), but was inhibited by a salt concentration of 120 g/L NaCl in the whey (pH 5.50); large variation in salt tolerance between strains was observed.

One of the potential difficulties to control *L.
monocytogenes in foods is the apparent salt resistance of the pathogen (up to 10% sodium chloride) (Pearson and Marth 1990). Since L. monocytogenes is present in white brined cheese in Turkey (Çiftçioglu and Uğur 1991; Bağcı 1997), the knowledge of its brine resistance is essential for determining the survival potential of this pathogen. There are many reports on the survival of L. monocytogenes in commercial cheese brines or in different solution containing sodium chloride (Papageorgiou and Marth 1989; Marth 1993; Larson et al. 1999; Miller et al. 1997). However, there is no study about the survival of L. monocytogenes in model brines at the different concentrations. Thus, the aim of this study was to determine the influence of brine with different salt ratios on L. monocytogenes in the model brines.

Materials and Methods

Inoculum preparation. L. monocytogenes serotype 4b used in this study (RSKK 475) was obtained from Refik Saydam National Type Culture Collection (Ankara, Turkey). The culture was maintained on tryptone soya agar (TSA, Oxoid, Basingstoke, UK) slants at 4 °C with bimonthly transfers and grown in tryptone soya broth (TSB, Oxoid) for 24 h at 35 °C. For inoculation in the brine, overnight cells of L. monocytogenes were pelleted by centrifugation (1600xg 30 min), washed three times and resuspended in the same volume of 0.1% peptone water (PW). The cells were diluted in PW to obtain the desired inoculum level before addition to the model brine.

Model brine preparation and inoculation. Brine samples were prepared by dissolving the appropriate quantity of NaCl in distilled water. Quantities of brines with different NaCl concentrations (13%, 15% and 19%, w/v) were dispensed into 250 mL flasks and autoclaved. Pasteurized whey (2% v/v) was aseptically added in the brines in order to reduce the acidity of the solution. Each brine flask was separately inoculated with L. monocytogenes 4b at the level of 10^7 CFU/mL and stored at 4 °C for 90 days. The duplicate samples were taken from three trials of the model brines after storage for 0, 2, 7, 15, 30, 60 and 90 days. Model brine samples containing 13%, 15% and 19% NaCl were abbreviated as B1, B2 and B3, respectively.

Microbiological analysis. Ten milliliters of samples were aseptically obtained from each samples and homogenized with 90 mL of 0.1% sterile peptone water for 2 min in stomacher. From this basic dilution, a series of decimal dilutions were prepared for microbiological analysis. Typical colonies of L. monocytogenes, which exhibited a black color, were enumerated by surface plating on Oxford agar (Oxoid) containing Listeria selective supplement (Oxoid) after an incubation period of 48 h at 35 °C. Five selected colonies were confirmed by streaking cultures onto TSA and isolated colonies were tested for the following characteristics: catalase production, tumbling motility at 25 °C, carbohydrate fermentation (maltose, dextrose, mannitol, xylose and rhamnose), nitrate reduction, Methyl-Red-Voges-Proskauer reactions, umbrella motility in SIM medium at 25 °C, β-hemolysis and Gram-staining (Hitchins 1995). The counting of L. monocytogenes was performed after storage for 0, 2, 7, 15, 30, 60, and 90 days until the organism was not detected by direct plating. If the organism was not detected by direct planting, then 25 mL of the samples added in 225 mL of Listeria enrichment broth (LEB, Oxoid) were enriched at 30 °C for 48 h and tested again for the presence of L. monocytogenes using the previously described procedures for planting on Oxford agar and confirming tests.

Statistical analysis. The data were analyzed using the SAS statistics package for windows (SAS/STAT Software 1998). Analysis of variance was applied to determine the existence of significant differences between the values. Significant (P<0.05) differences among means were identified using Duncan Multiple Range Test.

Results and Discussion

Brine samples were stored at 4 °C for 90 days and the pH of the brines was in the range of 5.65 to 5.78 during initial stage of storage. The survival of L. monocytogenes in model brines during storage was shown in Figure 1.

In brine B1, the number of L. monocytogenes remained relatively constant during the first 15 days. At 30th day of storage, the number of L. monocytogenes significantly (P<0.05) decreased and remained more or less constant throughout the rest of the storage. This result clearly demonstrated that L. monocytogenes survived for as long as 90 days in model brine B1.

In brine B2, population of the pathogen decreased significantly (P<0.05) until they became undetectable by direct planting at 30 days. The same samples gave positive results after enrichment throughout the storage (90 days).

A marked decrease (P<0.05) in the number of L. monocytogenes occurred in brine B3. L. monocytogenes decreased significantly (P<0.05) until they became undetectable by direct planting at 7 days. However, all samples were positive for L. monocytogenes after enrichment in LEB at this time period. The pathogen was not detected in this brine after 15 days of storage by both the direct planting and enrichment.

Papageorgiou and Marth (1989) studied the survival of L. monocytogenes in salted whey and found that the pathogen was able to grow in 6% salted whey, however, the pathogen was inhibited by 12% salted whey, which is
inconsistent with our results. On the other hand, Miller et al. (1997) reported that the \textit{L. monocytogenes} survived for 30 days at \(-12^\circ\text{C}\) in brine chiller conditions containing 20\% NaCl. The authors of above study indicated that low temperatures and high salt concentrations are not enough to prevent the survival of this pathogen. In another study, it was found that \textit{L. monocytogenes} inoculated into commercial cheese brines with NaCl content ranging from 5.6\% to 24.7\% survived for long times (ranged from <7 days to over 259 days), which has not been correlated with pH, salt content, nitrogen content, mineral content, or inherent microbial populations (Larson et al. 1999). The results of this study are not in agreement with the findings of the commercial brines reported above. This difference could be explained that because commercial cheese brines are mostly used repeatedly, the proteins and other nutrients from cheese are accumulated in brines, which make the brine a nutrient-rich environment for \textit{L. monocytogenes}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{The survival of \textit{L. monocytogenes} in model brines stored at 4 \(^{\circ}\text{C}\)}
\end{figure}

\begin{itemize}
\item B1: concentration of 13\% NaCl,
\item B2: concentration of 15\% NaCl,
\item B3: concentration of 19\% NaCl
\end{itemize}

\section*{Conclusion}
\textit{Listeria monocytogenes} is a halotolerant pathogen, resistant to the inhibitory effects of sodium chloride. This present study indicated that the \textit{L. monocytogenes} might survive in brines, if its salt concentration was not higher than 19\%. Therefore, any contamination of brines by \textit{L. monocytogenes} might be another contamination source for brined cheeses during ripening and storage. Therefore, it is recommended that the salting of cheese should be made from a brine containing 19\% NaCl and the brine should be stored for at least 15 days at 4 \(^{\circ}\text{C}\) in order to prevent survival of \textit{L. monocytogenes} before brining of cheese. Furthermore, the application of good manufacturing practices, especially proper sanitation of the cheese plant environment and a periodic antimicrobial treatment would seem to be necessary in preventing brine contamination by \textit{L. monocytogenes}.

\section*{References}

\textit{monocytogenes} from white pickled cheese consumed in Konya region, Ph.D. Thesis, Konya, Selçuk University, Health Services Vocational College (in Turkish).


