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The effect of cheese brine concentrations on survival of *Listeria monocytogenes*

Hisamettin Durmaz [1], Osman Aygun [2]* and Mustafa Ardic [1]

[1]*Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Harran University, 63300 Sanliurfa, Turkey.*
[2] *Programme of Food Technology, Suleyman Demirel Keban Vocational School, Firat University, 23700 Elazig, Turkey.*
**e-mail:oaygun@firat.edu.tr, hdurmaz@gmail.com, mardic@harran.edu.tr*

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Abstract

Although storage in brine is thought to cause a decrease in the populations of undesirable microorganisms, there is great concern that the brine can also serve as a reservoir for certain salt-tolerant pathogens. Therefore, 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. For this purpose, cheese brines containing 13%, 15% and 19% NaCl were inoculated with 10^3 *L. monocytogenes* (serotype 4b, RSKK 475) CFU/mL and stored at 4°C for 0, 15, 30, 60 and 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 plating at 30, 60 and 90 days gave negative results, the same samples gave positive results after enrichment. Number of *L. monocytogenes* in the 19% brine decreased faster than mentioned above for other salt concentrations and the pathogen was not detected in brine after 15 days of storage by both the direct plating and enrichment. The results showed that *L. monocytogenes* could survive in brines, if salt concentration was not higher than 19%. In conclusion, 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*.

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, inhibition of undesirable microorganisms, modification of flavour and texture and contribution to cheese ripening [6, 8, 12, 13]. 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 [1]. 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 for certain salt-tolerant pathogens. There have been a number of cases where especially brined cheeses have acted as carriers of foodborne infections [7], because poorly maintained brine tanks can become sources of contamination. Since *Listeria monocytogenes* can survive after the pasteurization process [4], 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, that makes the brine a nutrient-rich environment. Larson *et al.* [9] reported that *L. monocytogenes* survived for 118 days in fresh feta cheese brines (65 g/L NaCl and pH 6.8) at 4 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 [14] studied the fate of *L. monocytogenes* 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) [15]. Since *L. monocytogenes* is present in white brined cheese in Turkey [2, 3], 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 solutions containing sodium chloride [9-11, 14]. 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 (1600 x g, 30 min), washed three times and resuspended in the same volume of 0.1% peptone water. The cells were diluted in peptone water 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 10^3 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 B_1, B_2 and B_3, respectively.

Microbiological analysis: Ten millilitres 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 [5]. 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 plating, 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 [5].

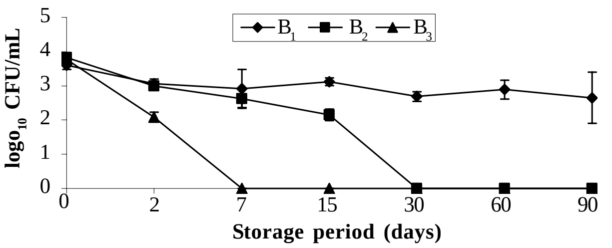
Statistical analysis: The data were analyzed using the SAS statistics package for Windows [16]. Analysis of variance was applied to determine the existence of significant differences between the values. Significant (P < 0.05) differences among means were identified using Duncan Multiple Range Test.

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 is shown in Fig.1. In brine B_1, 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 B_1.

In brine B_2, population of the pathogen decreased significantly (P < 0.05) until they became undetectable by direct plating 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 B_3. *L. monocytogenes* decreased significantly



B_1, B_2, B_3: Brine samples salted with 13, 15 and 19%, respectively.

Figure 1. The survival of *Listeria monocytogenes* in model brines stored at 4°C.

(P < 0.05) until they became undetectable by direct plating 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 plating and enrichment.

Papageorgiou and Marth [14] 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.* [11] reported that the *L. monocytogenes* survived for 30 days at –12° C in brine chilled 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, *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 [9]. 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 *L. monocytogenes.*

In conclusion, the present study indicated that *L. monocytogenes* might survive in brines, if salt concentration is not higher than 19%. It is recommended that firstly the salting of cheese should be made from a brine containing 19% NaCl and secondly the brine should be stored for at least 15 days at 4°C before brining of cheese in order to prevent survival of *L. monocytogenes.*

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