THE EFFECT OF DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE ON SURVIVAL OF *LISTERIA MONOCYTOGENES* IN WHITE SOFT CHEESE

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ABSTRACT

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The intrinsic characteristics of soft cheeses are perfect for L. monocytogenes growth because they are slightly acidic, have a high moisture content and a high water activity, and have a high fat content which can play a protective role for the organism against control treatments, and also because they contain high amounts of available nutrients. Thus the study aimed to determine the effect of different salt concentration (zero, 10%,15% and 20%, w/v) at different storage time (zero , 7 , 15, 30, 60 and 90) days on survival of *L. monocytogenes* artificially contaminated in manufactured soft white cheese. Results revealed that addtion of NaCl at concentration of 15 % for at least 30 days or 20% for 15 days during cheese manufacturing and storage at 4^{o} C could prevent survival of *L. monocytogenes* before consumption of cheese.

Keywords: *White cheese, L. monocytogenes, Sod.Chloride concentration.*

تأثير التركيزات المختلفة من ملح الطعام على حيوية ميكروب الليستيريا مونوسيتوجينز فى الجبن الأبيض الطرى

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يعتبر الجبن الأبيض الطرى مناسباً لنمو ميكروب الليستيريا مونوسيتوجينز حيث يتوافر به عدة عوامل مثل الحموضة الخفيفة، إرتفاع محتواه من الرطوبة، والدهون والتى تلعب دوراً هاماً فى حماية الميكروب من المعالجات المختلفة كذلك نتيجة لقيمتة الغذائية المرتفعة. أجريت هذه الدراسة للوقوف على تأثير إضافة ملح الطعام بتركيزات مختلفة (صفر،١٠ ،١٥ و٢٠%) عند فترات تخزين مختلفة (صفر،٧ ،١٥ ،٣٠ ،٦٠ و٩٠ يوما) على بقاء الميكروب. وقد أثبتت الدراسة أن إضافة ملح الطعام بنسبة ١٥% لمدة ٣٠ يوم أو ٢٠% لمدة ١٥ يوم أثناء تصنيع وتخزين الجبن عند ٤م^{o} كانت كافية لمنع بقاء الميكروب حيا حتى وقت استهلاكه. هذا وقد تم مناقشة الطرق الصحية لمنع تلوث الجبن بالميكروب حفاظا على صحة المستهلك.

INTRODUCTION

Listeria monocytogenes has become pathogen of concern for the food industry since documentation of its association with serious outbreaks of food borne illness (Schuchat *et al.,* 1991). *Listeria monocytogenes* multiplies over a wide range of temperature from 3 to 45° C and it is considered as psychrotolerant foodbrone pathogen. It grows over a pH range of 5 to 9.6 and salt concentration as high as 25.5% at 4° C and can cause infections in animals and humans and has been recognized as a significant foodborne pathogen for the past decade. The infections can be acute and severe causing meningitis with mortality up to30% in susceptible individuals as elderly, infants and immunocompromised people and can lead to abortion (Lund, 1990 and leuschner and Ilsch, 2003).

Foodborne illness associated with *L. monocytogenes* presents a major public health concern throughout the world (Hall, 1997). Major foodbrone outbreaks involved dairy products has been attributed to consumption of pasteurized milk (Fleming *et al.,* 1985), Mexican style soft cheese (James *et al.,* 1985), ice cream (Donnelly *et al.,* 1987) and Swiss regional type soft cheese (Bille and Glauser, 1988).

Raw milk can be contaminated either by L. monocytogenes from the dairy environment or during the milking process from mastitic udder. Contamination of soft cheese can occur during manufacture or post-production .The ubiquity of the organism and its ability to multiply in damp and cool conditions presents a problem in controlling post-production contamination even under good hygienic conditions (Harver and Gilmour 1992, Sanchz-rey *et al.,* 1993). Leuschner and Boughtflower (2002) found that if the milk was contaminated with L. monocytogenes before the cheese making process, it could survive the manufacture process and existed in the cheese at constant concentrations for up to four weeks.

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 flavor, texture and contribution to cheese ripening (Ibanez *et al.,* 1993; Laborda and Rubiolo 1999; Mullet *et al.,* 1999). Therefore, in cheese making processes of some traditional cheese varieties, a high salt content in brine is essential for controlling micro flora, preventing growth of pathogens and controlling enzyme activities during storage (Abd El-Salam *et al.,* 1993). Numerous studies have shown that the survival or growth of *L. monocytogenes* depends on the conditions during manufacture (Gameiro *et al.,* 2007). *L. monocytogenes* could survive in brine, if salt concentration was not higher than 19% (Durmaz *et al.*, 2009). The necessity of Sodium salts, particularly Sodium chloride, for the production of safe, wholesome foods and the key literature on the antimicrobial properties of sodium chloride in foods should be reviewed to address the impact of salt and sodium reduction or replacement on microbiological food safety and quality (Taormina, 2010).

This study was planned to study the effect of different concentrations of Sodium Cloride on survival of *L. monocytogenes* during manufacture and storage of soft white cheese

MATERIALS and METHODS

Inoculum preparation: L. monocytogenes (NCTC 7973/ATCC35152) used in this study was obtained from the reference strain bank of Food Hygiene Department-Animal Health Research Institute (AHRI), Doki, Giza. The strain was deep frozen stored in a cryoprotectant vial at (-30 ^{o} C). An inoculum of the pathogen was grown in Tryptic Soy Broth for overnight at 35^{o} C. Cells were centrifuged for 10 min at 8000 rpm. Supernatant was discarded and cells were washed three times and re-suspended in sterile 0.1% peptone water. The cells were (diluted in peptone water) adjusted to obtain the desired inoculum level (1-3x10^{9}) CFU/ml

before addition to the pasteurized milk for soft cheese production.

Preparation and inoculation: Cheese samples were prepared according to the traditional method by dissolving the appropriate quantity of NaCl in pasteurized milk. Different quantities of NaCl were added to obtain the required concentrations (zero, 10%, 15% and 20%, w/v), respectively and were dispensed into 1000 mL sterile beakers. Beakers were located in controlled water bath at 47^{o} C. Samples were separately inoculated by 1ml of *L. monocytogenes* inoculum to obtain a level of (10^{6} CFU/ml). Rennet was added to inoculated milk samples and were left in a water bath till the curd formation. Cheese curds were aseptically filtered from their original whey and added to previously prepared and autoclaved brine solutions (zero, 10%, 15% and 20%, w/v) respectively to put aside the effect of the contaminated cheese brine on survival of *L. monocytogenes* in the manufactured cheese. Cheese samples were stored at 4^{o} C for 90 days (experiment time) and were sampled at zero, 7, 15, 30, 60 and 90 Days. Samples containing Zero, 10%, 15% and 20% NaCl were abbreviated as C1, C2, C3, and C4, respectively.

Ten ml of milk samples of each NaCl concentration before curd formation and also ten gm of cheese samples from each NaCl concentration samples were obtained and homogenized with 90mL 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 according to APHA (2001). 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 according to (FDA, 2003) for the following characteristics:

Catalase production, carbohydrate fermentation (maltose, dextrose, mannitol, xylose and rhamnose), umbrella motility in SIM medium at 25°C, -hemolysis and Gram-staining. When the organism was not detected by direct plating, then 25 mL of the samples were added in 225 mL of Listeria enrichment broth (LEB, Oxoid), enriched at 30 ^{0}C for 48 h and retested for the presence of *L. monocytogenes* using the previously described procedures for plating on Oxford agar and confirming tests.

The results were statistically analyzed using SPSS for Windows version 16. "SPSS Inc. Headquarters, Chicago, Illinois USA.".

Table 1: Effect of sodium chloride concentration on the Survival of *L. monocytogenes* in white soft cheese.

Time \ Concentration	Zero	10%	15%	Detection	20%	Detection
Zero	7 x 10^{6A*}	6.6x10^{6A*}	4x $10^{6\ A*}$	ND	2.8 x $10^{6\ A*}$	ND
7day	6.4x$10^{6\ Ab*}$	6.2x10^{6Ab*}	4x$10^{4\ ab**}$	ND	1 x $10^{3\ B**}$	ND
15 day	1x10^{6Ab*}	6.5x10^{6Ab*}	2x$10^{3\ ab***}$	ND	Zero ^{B}	-ve
30 day	3 x $10^{7\ A*}$	5.8 x$10^{5\ a**}$	Zero	+ve	Zero	-ve
60 day	7.7 x $10^{6\ A*}$	5.4 x 0^{5a**}	Zero	+ve	zero	-ve
90 day	5.9 x 10^{6A*}	5.6 x 10^{5a**}	zero	+ve	zero	-ve

For rows: there is significant difference between cells have capital and its small letter
For columns: there is significant difference between cells have different numbers of (*)
ND: not done.
+ve, -ve: detection after enrichment

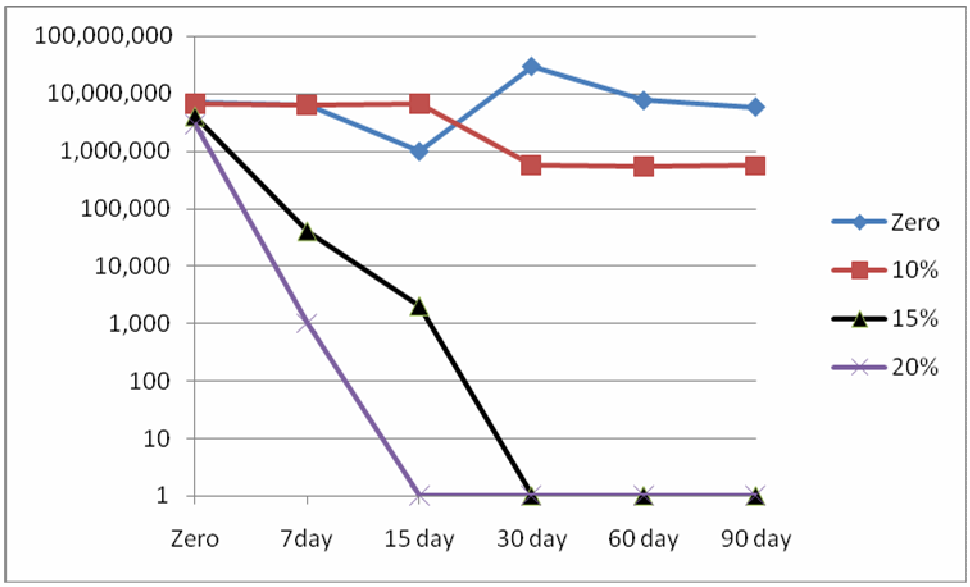

Fig. 1: Effect of sodium chloride concentration on the Survival of *L. monocytogenes* in white soft cheese

RESULTS

Results represented in Table 1 & Figure 1 showed the effect of different concentrations of NaCl on the viability of *L. monocytogenes* during ripening and storage of soft white cheese at 4°C.

It is worthy to mention that *L .monocytogenes* count in milk samples with different NaCl concentrations taken just after inoculation of the pathogen ranged from 1 - 4x10^6 CFU/g without any recorded significant differences. The population of *L .monocytogenes* at zero time (just after curd formation) in cheese samples with different NaCl concentrations were 7x 10^6, 6.6x 10^6 , 4x 10^6 and 2.8 x 10^6 CFU/g , respectively for the concentrations Zero (C1),10% (C2),15% (C3),and 20% (C4).

Although the pathogen counts apparently decreased by the gradual increase of the NaCl concentration, this decrease was insignificant.

Concerning Zero NaCl concentration (C1) it was observed that the population of the pathogen increased slightly from 7 x 10^6 to 3 x 10^7 CFU/g during storage and remained more or less constant throughout the rest of the storage time.

The addition of 10% NaCl (C2) had successfully reduced the contamination of *L .monocytogenes* by one log at the end of storage time (90 days). A significant decrease ($P<0.05$) in *L .monocytogenes* counts was recorded from C3 compared to (C2). While in samples (C3) there was a marked and significant decrease ($P<0.05$) in the pathogen count from 4x 10^6 to 4 x 10^4 and 2 x 10^3 at zero, 7th and 15th days of storage ,respectively until the pathogen failed to be detected by direct plating after 30 days storage. However, all samples were positive for *L. monocytogenes* after enrichment in LEB at the end of the storage period.

It was shown that the number of *L. monocytogenes* in (C4) was significantly decreased ($P<0.05$) from 2.8 x10^6 to zero on the 15th day but it could be detected by direct plating on 7th day (1x10^3 CFU/g). However the pathogen could not be detected at this concentration on the15thday of storage by both the direct plating & enrichment and thereafter.

DISCUSSION

The ability of *L. monocytogenes* to survive and grow at high salt concentrations and low temperature contributes to a potential health hazard after the consumption of contaminated

milk and dairy products and often involved in sever listeriosis outbreaks and constitutes a great challenge to the dairy industry.

Although the population of *L .monocytogenes* at zero time (just after curd formation) in cheese samples with different NaCl concentrations were apparently decreased by the gradual increase of the NaCl concentration , this decrease was insignificant ($P<0.05$). Meanwhile several investigators reported a significant drop in the pathogen count during cheese manufacturing procedures (Ryser *et al.,* 1985; Dominguez *et al.*, 1987; Kaufmann, 1990; Marth and Ryzer, 1990; Tawfik, 1993 and Hassan, 1996).

Inspite of the addition of 10% NaCl (C2) had successfully reduced the contamination of *L .monocytogenes* by one log by the end of storage time, it could not be relied on for its weak effect and long time onset (90 days). These results agreed with Larson *et al.* (1999) who reported that *L. monocytogenes* survived for 118 days in fresh feta cheese brines (6.5 % g/L NaCl) at 4 °C and 12 °C , it has been shown that *L. monocytogenes* can grow in salt solutions of up to 6% g/L NaCl. Many authors discussed that Sodium Chloride in concentration 1-7% did not inhibit the growth of *L .monocytogenes* (Pipova *et al.,* 2002). Moreover others stated that Cheese which made from raw milk with high salt level over 10% if contaminated with *L. monocytogenes* could be unsafe Papageorgiou and Marth (1989), Abdalla *et al.* (1993) and Hassan, (1996).

It is then strongly considered one of the potential difficulties to control *L. monocytogenes* in food because of the apparent salt resistance of the pathogen (up to 10% sodium chloride) (Pearson and Marth 1990).

For samples (C3) the obtained results showed a marked and significant decrease ($P<0.05$) in the pathogen count until the pathogen failed to be detected by direct plating after 30 days storage, meanwhile, all samples were positive for *L. monocytogenes* after enrichment which can be attributed to the partial injury of the cells with the increase of NaCl concentration and length of storage time.

In a work conducted by Papageorgiou and Marth (1989) to study the fate of *L. monocytogenes* in salted whey. The authors found that the pathogen was able to grow in salted whey (6% g/L), but was inhibited by a salt concentration of 12% g/L NaCl in the whey which is more or less inconsistent with the obtained results.

The extreme decrease of *L. monocytogenes* population while retaining its ability to be detected by direct plating till the 7th day and by enrichment until before the 15th day of storage at 4 ^{o}C in concentration as high as 20% (C4) was interestingly explained by other researchers as the environmental stresses such as low temperature and sodium chloride may lead to sublethally injured microorganisms. These sub-lethally injured microorganisms are viable, but they are physiologically deficient. Under favourable growth condition injured cells can repair and regain their pathogenicity (Ray, 1984).

In this concern, Durmaz *et al.* (2008) recorded that *L .monocytogenes* was destroyed at 19% NaCl concentration after7 days of storage at 4 ^{o}C. On the other hand, Miller *et al.* (1997) reported that *L. monocytogenes* survived for 30 days at –12°C in brine containing 20% NaCl. The authors indicated that low temperatures and high salt concentrations are not enough to prevent the survival of this pathogen. Also, Hefnawy and Marth (1993) examined survival of *L. monocytogenes* in different concentration of Nacl at 4° C and found that at this temperature *L. monocytogenes* grew in all NaCl concentrations tested but there is decrease in population occurred at 12thday of storage.

In another study carried out by Larson *et al.* (1999), it was found that *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). This result was explained that the commercial cheese brines are mostly used repeatedly, and the proteins

and other nutrients from cheese are accumulated in brines, which consequently makes the brine a nutrient-rich environment for *L. monocytogenes.* For this reason, In this study we replaced the formed curd in pre-autoclaved newly prepared brines in each time we repeated the experiment to put aside the effect of the brine accumulated nutrients or pathogen contamination on the survival of *L.monocytogenes* in different salt concentrations during storage time and to imitate as possible the traditional manufacture method and recipes to evaluate its efficiency in destruction of *L .monocytogenes.*

From the results mentioned above it is evident that the growth rate of *L .monocytogenes* was significantly decreased with increase of sodium chloride concentration and decrease of temperature of storage. In this regard other authors agreed with the obtained results that sodium chloride when exceeded 20%, survival of *L .monocytogenes* not exceeded 5 days (Marth, 1993).

In conclusion the results of this study highlighted that using different concentrations of NaCl (Zero %, 10%, 15% and 20%) during manufacturing of soft white cheese proved that the high level of NaCl concentration (20%) was completely effective in eliminating the pathogen from the experimentally contaminated cheese when stored at 4^{o}c for 15 days.

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