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THE SURVIVAL OF *LISTERIA MONOCYTOGENES* IN WHITE BRINED CHEESE

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L. monocytogenes survived for more than 40 days in all samples of white brined cheese examined. The numbers of L. monocytogenes in the cheese directly correlated with the total bacterial counts. In cheese kept at 4°C in 10% NaCl the number of L. monocytogenes increased from 1,2x10^5/g to 100x10^5/g to 100x10^5/g after 40 days, total bacterial counts decreased from 55x10^5/g to 8x10^5/g on day 28, and then increased to 370x10^5/g after 40 days. pH levels increased from 5,25 to 5,73. During storage of cheese at 4°C in brine with 16% NaCl, the number of L. monocytogenes increased in the first five days from 1,2x10^5/g to 39,5x10^5/g, decreased at 14 days to 6,5x10^5/g and then increased. In parallel to the change of L. monocytogenes counts total bacterial counts in the cheese slightly increased up to 14 days and then decreased and pH increased from 5,25 to 6,02. During the first 5 days of storage in brine with 10% NaCl at 8°C, L. monocytogenes counts increased from 1,2x10^5/g to 25x10^5/g and then slightly decreased to 4x10^5/g after day 40. In parallel to the change in number of L. monocytogenes in cheese, total bacterial counts increased from 55x10^5/g to 200x10^5/g on day 14 and then decreased to 32x10^5/g after day 40. The pH levels were increased from 5,25 to 5,59. In cheese kept in brine with 16% NaCl at 8°C L. monocytogenes counts increased from 1,2x10^5/g to 82,5x10^5/g on day 21 and then decreased. In parallel to the change of L. monocytogenes counts in cheese the total bacterial count increased during the first five days from 55x10^5/g, decreased to 71,5x10^5/g on day 14, was 17,5x10^5/g on day 28 and increased to 400x10^5/g at the end of storage. pH levels increased from 5,25 to 6,03.

Key words: L. monocytogenes, white brined cheese, survival, PH, total bacterial counts

INTRODUCTION

L. monocytogenes is a potential foodborne pathogen, widely spread in nature. This organism causes disease in idnividuals who have predisposing health conditions, such as the immunocompromised and pregnant women and their fetuses. In outbreaks of human listeriosis pasteurised milk (Fleming et al. 1985), mould ripened soft cheese (Bille and Glauser, 1988) and Mexican-style fresh cheese (Linnan et al. 1988) have been identified as vehicles of L. monocytogenes. L. monocytogenes can survive the processing of some dairy products (Ryser et al., 1989, Dominquez et al., 1987, Hicks et al., 1989). If it is present in foods L. monocytogenes can grow within quite wide ranges of pH, water activity and temperature. Refrigeration temperatures between 0-10^{0}C appear to be unable to prevent the growth of L. monocytogenes (Conner et al., 1986, George et al., 1988). At 4^{0}C, the doubling time of L. monocytogenes in raw naturally contaminated milk is 15^h, in artifically contaminated milk 18^h, in pasteurized artifically contaminated milk12^h and in Camambert chease 24^h. When soft cheese was kept for a long time, Listeria monocytogenes could be detected up to 10^5 and even 10^6 per gram (Beckers et al., 1987, Pini et al., 1988).

The purpose of this study was to determine the survival of L. monocytogenes in white brined cheese under different conditions of storage temperature and concentration of NaCl in the brine.

MATERIALS AND METHODS

Preparation of L. monocytogenes inoculum. The inoculum was prepared from 500 ml of a 24-hour L. monocytogenes (NCTC 7973) culture in brain heart infusion broth. The bacteria were pelleted by centrifugation (2000g for 20 min.) and resuspended in 50ml of sterile saline.

Inoculation of milk. Pasteurized whole (3,2% fat, pH 6,65) was contaminated with 10^5/ml of L. monocytogenes.

Manufacture and sampling of cheese. Pasteurized whole milk (76^{0}C, 15") was placed in a stainless steel pilot-plant sized stream jacked vat and warmed to 32^{0}C. Then, 0,8% starter (Streptococcus salivarius ssp. thermophilus and Lactobacillus delbrueckii ssp. bulgaricus) was mixed into the milk. After one hour 1ml of rennet was added per 5000 ml of milk and L. monocytogenes (10^5/ml). The renneting time was 45 minutes. The coagulum was cut with knives into pieces about 1,5-2cm^2 in size and gently stirred for about 20-30 minutes. After that the curd formed into a solid mas and the whey was run off. The cheese was stored at 4^{0}C in brine with 10% NaCl (Trial 1),eihter in 10% or 16% NaCl or in similar Strenths of brine respectively.

For enumeration of total bacterial counts and measurement of some pH cheese was produced in the some way without ading L. monocytogenes.

Determination of pH. The pH of the cheese was determined at the time of bacteriological analysis using a pH meter (pH meter Beckman).

Enumeration of L. monocytogenes. Duplicate samples of white brined cheese (20g) were dispersed in 180 ml 2% Na-citrate. Decimal dilutions of cheese extract were plated on to lithium chloride-phenylethanol-moxalactam agar. The plates were incubated in air at 37^0C for 48^h and typical colonies were counted and confirmed as described by Skogaard and Morgan (1988).

Enumeration of total viable bacterial count. The total bacterial counts in white brined cheese were determined in decimal dilutions of milk in sterile physiological saline on total bacterial count agar plates (Torlak-Yugoslavia) which were incubated at 30-32^0C for 48^h.

RESULTS

The results concerning L. monocytogenes survival in white cheese, stored at 4^0C in brine for 40 days are shown in Table 1.

Table 1. Change in L. monocytogenes counts, total bacterial counts and pH levels during storage of cheese at 4^0C

Aga of cheese (days)	Trial 1 (10% NaCl)			Trial 1 (16% NaCl)		
	L. monocyt. (10^5/g)*	TBC* (10^5/g)	pH*	L. monocyt. (10^5/g)*	TBC* (10^5/g)	pH*
0	1,2	55	5,25	1,2	55	5,25
5	10	36,5	5,52	39,5	19,5	5,60
14	37,5	16,5	5,69	6,5	60	5,93
21	67	11,6	5,84	14,1	58	6,00
28	66	8	5,90	48	6	6,00
40	100	370	5,73	43	600	6,02

*Mean values of three determinations
TBC-Total bacterial counts (CFU/g)

In the cheese stored in brine with 10% NaCl at 4^0C, L. monocytogenes survived for 40 days and the number of L. monocytogenes increased from $1,2x10^5$/g to $100x10^5$/g. In parallel to the change of L. monocytogenes counts, total bacterial counts decreased from $55x10^5$/g to $8x10^5$/gon day 28 and then increased up to $370x10^5$/g, while pH levels increased from 5,25 to 5,90 on day 28 and then decreased to 5,73. In cheese kept at 4^0C in brine with 16% NaCl the number of L. monocytogenes increased in the first five days from $1,2x10^5$/g to $39,5x10^5$/g, and then decreased to $6,5x10^5$/g at 14 days and then increased. In parallel to the change of L. monocytogenes counts in cheese the total bacterial count slightly increased in the first five days, was lower at 14 days and then increased. The pH levels increased from 5,25 to 6,02.

The survival of L. monocytogenes in white brined cheese during storage at 8^0C is shown in Table 2.

Table 2. Change in L. monocytogenes counts, total bacterial counts and pH levels during storage of cheese at 8°C

Aga of cheese (days)	Trial 2 (10% NaCl)			Trial 2 (16% NaCl)		
	L. monocyt. (10^5/g)*	TBC* (10^5/g)	pH*	L. monocyt. (10^5/g)*	TBC* (10^5/g)	pH*
0	1,2	55	5,25	1,2	55	5,25
5	25	94,6	5,50	49	95	5,60
14	12,5	200	5,75	57,5	71,5	5,93
21	5,5	120,5	5,81	82,5	60	6,05
28	3,3	58	5,86	42	17,5	6,00
40	4	32	5,59	12	400	6,03

* Mean values of three determinations
TBC-Total bacterial counts (CFU/g)

In the first 5 days of storage in brine with 10% NaCl at 8°C L. monocytogenes counts increased from 1,2x10^5/g to 25x10^5/g and then slightly decreased to 4x10^5/g on day 40. In parallel to the change of L. monocytogenes counts in cheese total bacterial counts increased from 55x10^5/g to 200x10^5/g on day 14 and then decreased to 32x10^5/g after day 40. During cheese storage pH levels rose from 5,25 at the beginning of the study to 5,86 on day 28 and then decreased to 5,59. The results for cheese, kept in brine with 16% NaCl at 8°C, showed that L. monocytogenes counts increased from 1,2x10^5/g to 82,5x10^5/g on day 21 and then decreased. In parallel to the change of L. monocytogenes counts the total bacterial count increased in the first five days from 55x10^5/g to 95x10^5/g, decreased to 71,5x10^5/g on day 14, was 17,5x10^5/g on day 28 and increased to 400 x10^5/g at the end of the storage. During cheese storage the pH level increased from 5,25 at the beginning of the study to 6,03 at the end.

DISCUSSION

The results show that L. monocytogenes survived during manufacture of white brined cheese and storage at 4°C and 8°C in 10% and 16% NaCl. The increasing L. monocytogenes count, during storage was directly related to the total bacterial count. During storage of cheese in brine with 10% NaCl at 4°C the increase of L. monocytogenes counts was higher than in the cheese kept at 8°C. The increase in total bacterial counts in the cheese kept at 8°C was higher than in the cheese kept at 4°C. The L. monocytogenes count in the cheese kept at 8°C in brine with 16% NaCl was higher than in the cheese kept at 4°C in brine with 16%. Thus, the rate of growth of L. monocytogenes in cheese depends on the ecological conditions (water content, pH, temperature, salt content). Beckers et al. (1987) and Pini et al. (1988) found that L. monocytogenes could be detected in samples of soft cheese up to 10^5/g and even 10^6/g. Štajner et al. (1979) found that when unsalted cheese was prepared from naturally-infected skim milk containing 5x10^5 L. monocytogenes/ml the organism survived through 7 days of storage at 3-5°C. If L. monocytogenes is

present in Feta-cheese it may survive during storage for many weeks in the cheese as well as in the brine (Terplan, 1988).

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PREŽIVLJAVANJE *L. MONOCYTOGENES* U BELOM SIRU U KRIŠKAMA

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SADRŽAJ

L. monocytogenes je preživljavala u svim uzorcima belog sira u kriškama duže od 40 dana. Broj L. monocytogenes u siru direktno zavisi od ukupnog broja mikroorganizama. U siru čuvanom na 4°C u 10% presolcu broj L. monocitogenes se povećavao sa $1,2x10^5$/g na $100x10^5$/g posle 40 dana, ukupan broj bakterija se smanjivao sa $55x10^5$/g na $8x10^5$/g posle 28 dana, a 40-og dana se povećavao na $370x10^5$/g, pH se povećavao sa 5,25 na 5,73. Za vreme čuvanja sira na 4°C u 16% presolcu broj L. monocytogenes se u prvih pet

dana povećavao sa 1,2x10^5/g na 39,5x10^5/g, smanjivao posle 14 dana na 6,5x10^5/g a potom povećavao. Paralelno sa promenom broja L. monocytogenes u siru ukupan broj bakterija se neznatno povećavao do 4 dana, a potom se smanjivao, pH se povećavao sa 5,25 na 6,02. U prvih pet dana čuvanja sira u presolcu sa 10% NaCl na 8^{0}C broj L. monocytogenes se povećavao sa 1,2x10^5/g na 25x10^5/g, zatim se neznatno smanjivao i 40-og dana iznosio 4x10^5/g. Uporedo sa promenom broja L. monocytogenes u siru ukupan broj bakterija se povećavao sa 55x10^5/g na 200x10^5/g četrnaestog dana, a zatim se smanjivao na 32x10^5/g posle 28 dana, pH se povećavao sa 5,25 na 5,59. U siru čuvanom u presolcu sa 16% NaCl na 8^{0}C broj L. monocytogenes se 21 dana povećavao sa 1,2x10^5/g na 82,5x10^5/g, a zatim se smanjivao. Uporedo sa promenom broja L. monocytogenes ukupan broj bakterija se povećavao u prvih pet dana sa 55x10^5/g na 95x10^5/g, a zatim se smanjivao na 71,5x10^5/g 14-0g dana, 17,5x10^5/g 28-og dana, a 40-og dana se povećao na 400x10^5/g. pH se povećao sa 5,25 na 6,03.