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INFLUENCE OF STORAGE TEMPERATURE ON VIABILITY OF *LISTERIA MONOCYTOGENES* AND *SALMONELLA TYPHIMURIUM* IN WHITE SOFT CHEESE

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SUMMARY

The present experiment was carried out to study the effect of storage temperatures of white soft cheese on the survival and growth of *Listeria monocytogenes* and *Salmonella typhimurium*. *L. monocytogenes* showed slight decrease in viable cell count during the first few days of refrigerator storage (8.0 log CFU/g) followed by stationary period (4 weeks). The detectable cells increased gradualy, near the end of the experiment which lasted for about 4 months, then the viable cell count decreased gradually again. Whereas at room temperature the organism exhibited slight decrease in number (0.4 log CFU/g) followed by short stationary period, then the viable cell count increases gradually but slowly till the end of the experiment (20 days) at which the product showed signs of spoilage.

S. typhimurium appeared to be a microorganism of normal behavior. It showed decrease in number directly after storage by 0.3 and 0.9 Log CFU/g, followed by stationary phase for about 28 and 12 days, finally the organism tend to decrease gradually until the end of the experiment (65 and 36 days) for samples stored at 4°C and room temperature respectively due to the spoilage of the cheese.

Public health importance and suggested control measures of the examined organisms were discussed to improve the keeping quality of the white soft cheese.

INTRODUCTION

White soft cheese is one of the most popular dairy products which is widely used all over the world due to its high nutritive value and its palatability to consumers. However, it may constitute serious public health hazards due to contamination with various food-borne pathogens during different steps of manufacture, storage and distribution.

L. monocytogenes has become a pathogen of concern for the food industry since documentation of its association with several serious outbreaks of food-born illness. The organism become one of the most studied causes of food poisoning in the last 10 years because of its ability to grow at refrigeration temperature and because of the serious illness that it can causes specially in immunocompromised individuals (Schuchat, et al., 1992 and Yeu-Hsin and Donnelly, 1992). Moreover, recent outbreaks of food-borne listeriosis have generated much interested in defining the behavior of *L. monocytogenes* in food system (Bradshaw et al., 1985 and Doyle et al., 1985).

S. typhimurium inhibits the gastrointestinal tract of all warm-blooded animals, including humans, thus food of animal origin particularly those susceptible to fecal contamination are likely to serve as sources of Salmonella (Bradshaw et al., 1987). Salmonellosis is characterized by onset of fever, diarrhea and vomiting within 24 to 48 hour following consumption of contaminated food, fortunately mortality rate is low but may be high for infants and elderly (Chalker and Blaser, 1988).

Microorganisms that have experienced environmental stresses such as heating, freezing and exposure to acids can become sub-lethaly injured (Beuchat et al., 1986, Buchanan et al., 1987; Bunning et al., 1988; Golden et al., 1988a and Smith and Archer, 1988). In the injured state, bacteria become sensitive to agents to which they would otherwise show resistance, although injured cells lose disease producing capacity, these bacteria can regain the capacity to multiply under favorable growth conditions (Ray, 1979 and Jay, 1986).

Since white soft cheese constitute an essential part of human diet in Egypt and awing to the little information available , that defines the effect of stress factors during procesing and storage of cheese, thus the aim of this study is to determine the behavior of *L. monocytogenes* and *S. typhimurium* in while soft cheese during storage either at room and refrigeration temperatures.

MATERIALS AND METHODS

1. Organism and growth codnitions:

L. monocytogenes and S. typhimurium strains were provided by the Federal Institute for Helath protection of Consumers and veterinary Medicine, Berlin, Germany. Cultures were prepared following the directions described by ATCC (1992) using two successive 24 hours incubation at 35°C in Trypticase Soya Broth with 0.6% Yeast extract.

2. Preparation of the white soft cheese:

White soft cheese was manufactured in the laboratory from Listeriae and Salmonellae free milk. The milk was artificially infected separately with a suspension of 24 hours *L. monocytogenes* and *S. typhimurium*. The cheese was obtained by rennin coagulation according to

the procedure described by Fahmi and Sharara (1950). The prepared cheese was microbiologically examined to detect the initial bacterial count (6.9 and 6.6 Log CFU/g for *L. monocytogenes* and *S. typhimurium* respectively). Then the cheese blocks with whey were divided into two portions; the first was kept in refrigerator (4°C) while the second portion was stored at room temperature (about 25°C).

3. Design of the experiment:

Two trials plan was designed to assess the effect of storage temperature of the white soft cheese on the growth and survival of *L. monocytogenes* and *S. typhimurium*.

4. Sampling and monitoring of the organisms:

A duplicate 10 grams from each sample were obtained at appropriate intervals and prepared for enumeration of:

4.1. *L. monocytogenes:* by surface plating onto Modified Oxford Agar. Plates were incubated at 37°C for 48 hours, where the typical colonies of *L. monocytogenes* were counted (APHA, 1992).

4.2. *S. typhimurium:* by surface plating onto S. S agar, Plates were incubated at 37°C for 24 hours, where the typical colonies of *S. typhimurium* were counted (APHA, 1992).

5. Statistical analysis:

Average of the counts (Log CFU/g) were recorded, before all data were subjected to stais-tical analysis.

RESULTS AND DISCUSSION

All obtained data from the viability study of both *L. monocytogenes* and *S. typhimurium* in white soft cheese stored at room and refrigerator temperature was illustrated in tables 1 & 2 and figures 1-4.

The viable cell count of *L. monocytogenes* was significantly decreased during the first few days of storage from 6.9 to 6.1 and 6.5 Log CFU/g in curd at refrigerated and room temperature, and from 9.2 to 8.5 and 8.1 in whey. Then the viable cells showed a constant phase of growth for about 4 weeks which was more evident at refrigerator than at room temperature (at which the product showed signs of deterioration), followed by another pattern of behavior after about 40 days at refrigerator where the viable cells increase gradually by about 0.5 intervals Log CFU/g in both curd and whey and lasted about 90 days. Finally, at the end of the experiment the viable Listeria count decreases gradually until it reach 2.4 and 1.6 Log CFU/g in curd and whey at refrigeration storage respectively (Table 1 and Fig. 1 & 2).

The special behavior of *L. monocytogenes* obtained in these study could be explained on the

basis of exposure of the organism to different stress factors as increased acidity, sodium chloride and the ripening process itself which may lead to injury of cells (Dominguez et al., 1982; Conner et al., 1986; Ahamad and Marth, 1988; Kaufman, 1990; Donnelly, 1992; Tawfik, 1993; Rajkowski et al., 1994). Moreover, Golden et al., (1988b); Flanders and Donnelly (1994) and Hassan (1996) found that injured Listeria may lose the disease producing capacity as well as escape detection with the normal isolation procedures, however under favorable growth conditions, they may repair the sub-lethal injury and regain the capacity to multiply. In addition Conner et al., (1986) and Donelly (1992) stated that *L. monocytogenes* is psychrotrophic in nature that have the ability to multiply over a wide range of temperatures between 3 and 45°C.

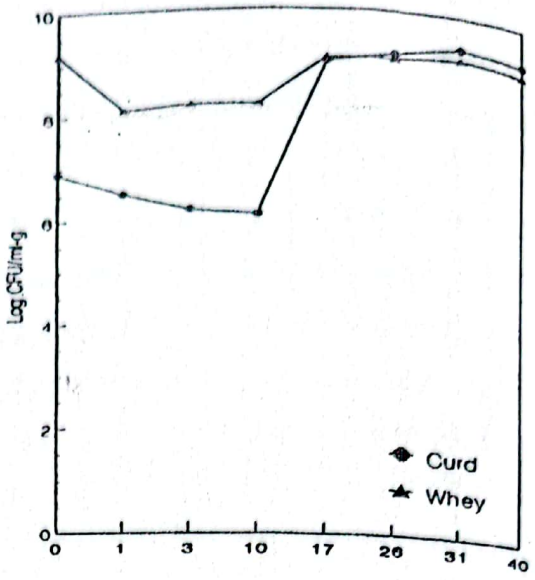

Fig. (2): Influence of storage at room temperature on viability of *L.monocytogen* in white soft cheese.

Table (2) and Fig. (3) and (4) revealed the behavior of *S. typhimurium* in white soft cheese during storage period. The initial population was 6.6 Log CFU/g which decreased by 0.6 and 0.8 Log CFU/g in curd and whey at °4C and by about one Log in both curd and whey at room temperature, this

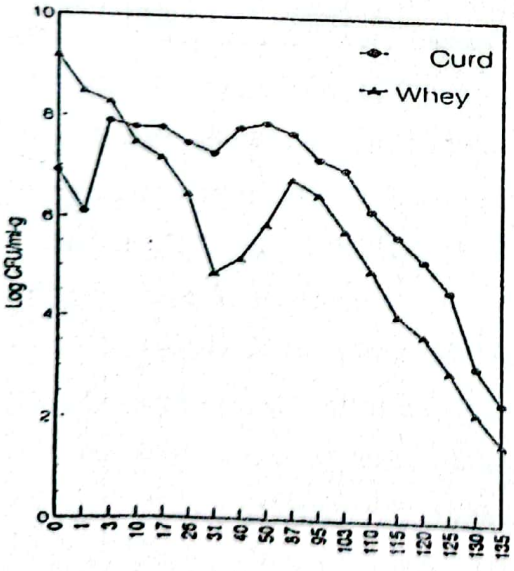

Fig. (1): Influence of storage at 4°C on viability of *L. monocytogen* in white soft cheese.

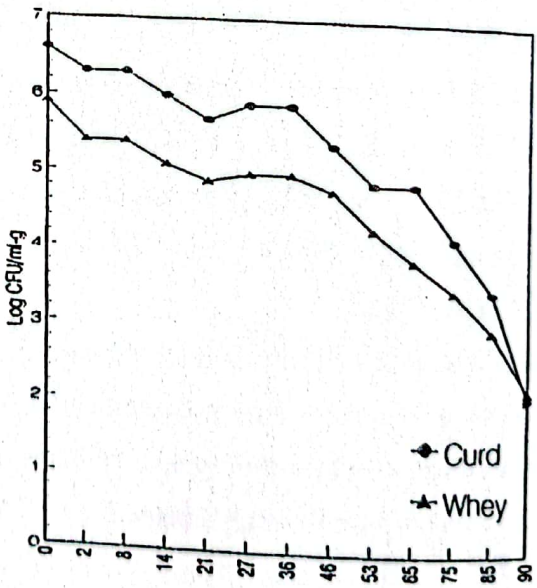

Fig. (3): Influence of storage at 4°C on viability of *S. typhimurium* in white soft cheese.

period lasts for about 2 weeks. After that the viable cell count depict a period of constant growth that lasted for about 46 days at refrigeration and 21 days at room temperature. Finally the viable cells started to decrease in number gradually until it reaches about 2.1 Log CFU/g in both curd and whey at refrigerator storage after about 90 days, while it reaches about 4.8 Log CFU/g at room temperature after about 46 days at which the product showed signs of deterioration.

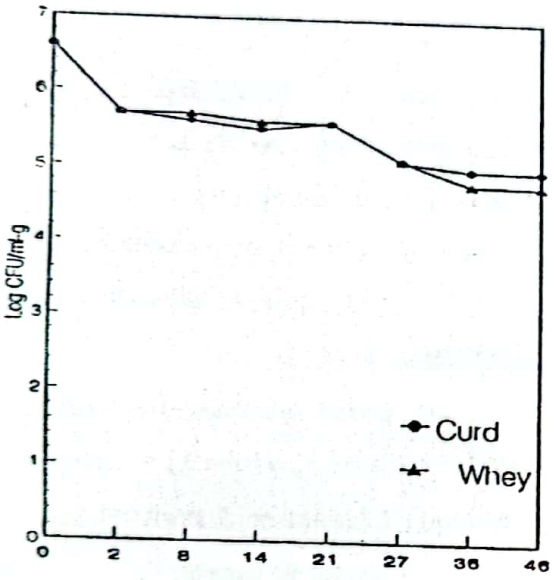

Fig. (4): Influence of storage at room temperature on viability of *S. typhimurium* in white soft cheese.

The behavior of *S. typhimurium* may be explained by the fact that Salmonella organisms is a delicate one which can not survive the different processing treatments during cheese preparation. It was commonly known that incidence of Salmonellae in dairy products is usually low due to the adverse condition it was subjected during production (Bradshaw et al., 1987).

In conclusion, the use of pasteurized milk, prevention of post-treatment contamination and application of good manufacturing and storage practices are extremely important to safe cheese production, also it is necessary to understand the pattern of microbial growth and the nature of the

Table (1): Effect of storage temperature at room and 4°C on viability of *L. monocytogenes* in white soft cheese.

Days (days)	Cell count Log CFU/g			
	Room Temperature		4°C	
	Curd	Whey	Curd	Whey
0	6.90	9.20	6.90	9.20
1	6.50	8.10	6.10	8.50
3	6.20	8.20	7.90	8.30
10	6.10	8.20	7.80	7.50
17	9.03	9.10	7.80	7.20
26	9.20	9.10	7.50	6.50
31	9.40	9.20	7.30	4.90
40	9.30	9.10	7.80	5.20
50	*	*	7.90	5.90
87	*	*	7.70	6.80
95	*	*	7.20	6.50
103	*	*	7.00	5.80
110	*	*	6.20	5.00
115	*	*	5.70	4.10
120	*	*	5.20	3.70
125	*	*	4.60	3.00
130	*	*	3.10	2.20
135	*	*	2.40	1.60

Table (2): Effect of storage temperature at room and 4°C on viability of *S. typhimurium* in white soft cheese.

Days (days)	Cell count Log CFU/g			
	Room Temperature		4°C	
	Curd	Whey	Curd	Whey
0	6.60	6.60	6.60	5.90
2	5.70	5.70	6.30	5.40
8	5.60	5.70	6.30	5.40
14	5.50	5.60	6.00	5.10
21	5.60	5.60	5.70	4.90
27	5.10	5.10	5.90	5.00
36	5.00	4.90	5.90	5.00
46	5.00	4.80	5.40	4.80
53	*	*	4.90	4.30
65	*	*	4.90	3.90
75	*	*	4.20	3.50
85	*	*	3.50	3.00
96	*	*	2.10	2.20

product under preparation to minimize the risk of life threat arise from consumption of these products.

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