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GROWTH AND SURVIVAL OF *E. coli* AND *S. Typhimurium* IN CULTURED BUTTERMILK

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ABSTRACT

This study was carried out to investigate growth and survival patterns of *E.coli* or *S.typhimurium* before and after fermentation process of cultured buttermilk product. Emphasis was given to evaluate the effect of level of inoculation, stage of inoculation (pre- or post- fermentation) and the storage temperature on the survival of both bacteria. When *E.coli* at two levels (6 or 2 logCFU/ml) was inoculated separately into the milk before fermentation, an increase in the bacterial count of 1.5-3 folds was obtained for the two inoculum's sizes and after 6, 12 hr of fermentation period respectively. The count then declined gradually for each when the fermented milks were stored at 25 or 5^{o}C although the reduction was faster in all cases at 25^{o}C than that at 5^{o}C reaching undetectable levels within 18 and 30 days at two forementioned temperatures respectively for the high inoculum's size. In contrast, samples with low *E.coli* inoculum showed a relatively fast disappearance of bacterium as it became undetectable after just 16 and 25 days of storage at 25 and 5^{o}C respectively.

A continuous decline was observed when *E.coli* was inoculated directly into the fermented product stored at two tested temperatures with rapid disappearance at 25^{o}C (16 days) compared with that at low one (5^{o}C) (21days) for high inoculum's size (6.1 log CFU/ml) whereas lowest survival (3days) was reported with low inoculum size (2.4 log CFU/ml). *S.typhimurium* showed nearly the same patterns of growth that observed with *E.coli* when pathogen was inoculated before fermentation. The lowest survival of *S.typhimurium* was encountered when it was inoculated directly in the fermented products as it disappeared after few days (2 or 3 days) of storage at 25 and 5^{o}C respectively. In light of accumulated evidence, safety and keeping quality of cultured buttermilk were more related to stage of contamination and storage temperatures of final product.

INTRODUCTION

The presence of coliform bacteria in dairy products is generally provides an index of the hygienic standard of the products and also of its keeping quality (Potasknik *et.al*.,1972). Recently there has been additional concern about the presence of *E.coli* in fermented dairy products because of the ability of some strains to cause foodborne illness, Dineen, *et al.* (1998). Isolation of *E.coli* O157:H7 from patients whose illness were epidemiologically linked to the consumption of contaminated fermented foods (Morgan *et al*., 1993; CDC, 1995; Hudson *et al*., 1997) and the isolation of some other pathogens (Gran *et.al*, 2003) and *Salmonella* (Fontaine, *et al*., 1980) from these products represent a potential health hazard which might be caused by consuming the contaminated fermented products. In addition growth of *E.coli* in dairy products can cause defects in flavor and texture (Frank and Marth, 1977). In Libya fermented dairy products industry is growing fast and cultured buttermilk which known locally as "Laban" is a leading product of this industry and one of the widely consumpted fermented product in the country. Because cultured buttermilk is of that commercial

significance and no study was conducted in Libya to determine the behavior of *E.coli* or *S.typhimurium* in the local cultured buttermilk, this study was undertaken to determine the growth and the survival of *E.coli* and *S.typhimurium* as pre- and post-fermentation contaminants in cultured buttermilk at different points of processing and storage.

MATERIALS AND METHODS

Strains and culture conditions. Culture of *E.coli* No.43.3 kindly supplied from Biotechnological Researches Center, Tripoli-Lipya and *S.typhimurium* (obtained from the Department of Microbiology and Animal Health Research Institute, Cairo, Egypt) were grown twice separately overnight with shaking (200 rpm) at 37^{o}C in nutrient broth (Plasmatce Laboratory Products, Bridport, UK) before use. Appropriate amounts of each culture (culture incubated for 18-24 hrs contained about 10^{9} CFU/ml) or their serial dilutions were added to milk samples to meet the desired cell density.

Dri-Vac mesophilic culture (Hansen CHN-11) which is usually used in Libya to manufacture this product and consisting of *Lactococcus lactis* subsp*. cremoris* , *lactococcus lactis* subsp*.lactis, leuconostoc citrovoum and lactococcus lactis* subsp *diacetylactis* was reactivated as described by Robinson, (1981).

Cultured buttermilk preparation

In attempts to imitate the industrial conditions buttermilk samples were prepared under laboratory conditions with similar compositional and processing parameters. Milk composition, buttermilk culture, processing were maintained as close as possible to the commercial conditions of the product as they prevail in the country. Briefly and as described by Robinson, (1981) reconstituted skimmed milk of 10% total solids was prepared from instant commercial skimmed milk powder. The milk was heated (85-90^{o}C) for 30min. then cooled down to 21^{o}C and inoculated with 1% (g/v) of Dri-Vac mesophilic culture. Milk samples were then incubated at 21^{o}C for 18-20hrs.

Inoculation with *E.coli* or *S.typhimurium*.

To determine the effect of the inoculum's stages (pre- or post-fermentation) and the storage temperatures on the survival of *E.coli* or *S.typhimurium* in the cultured buttermilk the tested microorganisms were added separately either before or after the fermentation process. Milk samples to be made into cultured buttermilk and final buttermilk product were inoculated with *E.coli* or *S.typhimurium* at two inoculum's levels each. Two levels of inocula were chosen in each case to represent low and high cell density of possible product contamination. After heat treatment at ~90^{o}C for 30min, cooling to 21^{o}C and just before addition of the lactic acid culture the milk samples were inoculated separately with two levels of *E.coli* culture (10^{6} or 10^{3} CFU/ml of milk) or *S.typhimurium* (~ 5 log CFU/ml or 3 log CFU/ml) by the addition of the proper amounts of nutrient broth previously inoculated with each bacterium to milk samples. Inoculated milk samples were mixed thoroughly and inocubated at 21^{o}C for 18-20hrs. At various time intervals, milk samples of each case were withdrawn and analyzed immediately in duplicate for *E.coi* or *S.typhimurium* and pH values during fermentation

period (20-24hrs). The fermented milk samples were then stored at either 5± 1°C or ~25°C to study the effect of the storage temperatures on the survival of tested bacteria. To investigate the survival patterns when each bacterium was inoculated directly into fermented milk samples, another cultured buttermilk samples were prepared and within 24hrs of preparation the final products were inoculated with *E.coli* or *S.typhimurium* in the same manner previously described. Inoculated fermented milk samples were then divided into two groups: one group was stored at 5°C ±1 while the other kept at ambient temperature (~25°C) for up to 30 days. Samples were taken periodically and the bacterial counts were determined along with pH values until the bacterium became undetectable (<10 CFU/ml).

Enumeration of *E.coli* and *S.typhimurium*:

Samples of each treatment were taken at 0, 6, 12, and 24hrs in the first day of processing then periodically up to 30 days (at 5 or 25°C) for the pre-fermentation inoculated milk samples. Samples of cultured milk inoculated after fermentation process (stored at either 5 or 25°C) were taken at 0, 24, 72 hrs. and periodically until the bacterium became negligible(<10 CFU/ml). Samples were serially diluted in buffered peptone water (Merck KGaA, Darmstadt,Germany) plated in duplicate on MacConkey agar (Difco,1969) and incubated overnight at 37°C for 24hrs. *S.typhimurium* was plated on Salmonella Shigella Agar,(S.S.A) (Difco,1969) and incubated at 37°C for 24-48hrs then colonies were counted.

pH measurements:

pH measurements were determined with pH meter (A-S 502, UK) equipped with a glass electrode.

Statistical analysis: the data were analyzed using Excel Microsoft.

RESULTS AND DISCUSSION

Survival of *E.coli* in skimmed milk during fermentation

The fate of *E.coli* in milk after addition of buttermilk culture was monitored during 24 hrs of fermentation process and periodically there after up to 30 days until the *E.coli* became negligible (less than 10 CFU/ml). The results in (Table 1) indicate an increase in *E.coli* counts during the initial stages of fermentation at the two levels of inoculum (6 and 2 log CFU/ml) tested (Table 1). The highest increase at the first level (8 log cfu/ml) was reported after 6 hr of fermentation whereas the count of *E.coli* reached a maximum of 6.6 log cfu/ml for lower inoculum after 12 hrs of fermentation process then the count started to decline thereafter (Table1). These results agree with those obtained by Mufandaedza *et al*., (2006) who reported that *E.coli* survived and grew and reached high populations of about 9 log CFU/ml, after 18hrs when it was inoculated at the beginning of the fermentation. Also, Frank and Marth (1977) found that *E.coli* cells were able to increase in number as much as three log cycles during initial stage of milk fermentation. In the present study the pH values for both inoculum's sizes declined significantly ($P<0.05$) during fermentation period and ranged from 4.3 to 4.4 at the end of fermentation process.

Effect of storage temperatures on the behavior of *E.coli*

Beyond 20-24 hrs of the incubation at 21°C, at the end of fermentation process , the fermented milks of each *E.coli* inoculum size were then stored at either 5 or 25°C for up to 30 days to determine if there is any effect of the storage temperature on the behavior of *E.coli* bacterium. In general longer *E.coli* survival was observed at refrigerated temperature than at ambient temperature for both inoculum's sizes of bacterium. As can be seen in (Table 2) when the fermented milks with high *E.coli* inoculum were stored at ambient temperature (~25°C) or 5°C a significant decrease ($P<0.05$) in the *E.coli* counts were reported during storage period of 30 days reaching 1.2 and 2.9 log CFU/ml after nearly two weeks (16 days) of storage at forementioned temperatures respectively to became no longer detected (<10 CFU/ml) after 18 days (25°C) and 30 days (4°C) of storage period. However fermented milk with low inoculun size stored at ambient temperatures showed a rapid decrease in the bacterial count as no *E.coli* was recovered after only 16 days of storage (Table 2). These findings were supported by the results obtained by Frank and Marth, (1977) who found that the longest survival time for *E.coli* in refrigerated fermented milk was about 17 days when milk was inoculated with the bacterium (10^3 CFU/ml) and fermented at 32°C with 0.25% lactic starter. In present study long persistence of *E.coli* in fermented product produced by inoculated milk before fermentation was attributed to the reduction of the initial shock of acid condition to the cell and thus allow for long survival of the bacterium (Cheng Hsin and Cheng-Chun 2001). Survival of *E.coli* in refrigerated fermented dairy products has been studied previously (Goel *et al*., 1971; Potashnik *et al*., 1972; Park *et al*.,, 1973) and found to be dependent on storage temperature, type of product, type of lactic bacteria used and pH of the products. Data presented in (Table 2) show no significant difference ($P > 0.05$) was reported in pH values which were kept nearly steady during storage period and ranged from 4.3 to 4.5 (Table 2).

Table 1: Growth and survival of *E.coli* in milk during fermentation to cultured buttermilk [a]

1st inoculum (~ 6 log CFU/ml)			2nd inoculum (2 log CFU/ml)	
Time (hr)	**pH [b]**	**count [c]**	**pH**	**count**
0	6.5 ± 0.06	5.9 ± 0.1	6.5 ± 0.0	2 ± 0.0
6	5.2 ± 0.15	8.0 ± 0.3	6.3 ± 0.40	6.0 ± 0.3
12	4.5 ± .03	7.0 ± 0.0	4.5 ± 0.10	6.6 ± 0.5
24	4.3 ± 0.06	6.8 ± 0.1	4.4 ± 0.03	6.0 ± 0.3

[a] Post-heated milk samples were inoculated separately with two levels of *E.coli* just before addition of starter cultures and incubated at 21°C for 20hr.
[b]All pH values and *E.coli* counts are the mean of two samples each done in duplicate.
[c]count: Log CFU/ml.

Survival of *E.coli* in fermented milk

The survival of *E.coli* bacterium in fermented milk as a result of contamination at the final stages of cultured buttermilk processing was monitored at tow inoculum's levels. When *E. coli* was inoculated into previously fermented milk at high inoculum level (6.1 log CFU/ml), the viable count of *E.coli* showed a graduate decrease in numbers during the storage

period at either 5±1 or 25±2°C (ambient temperature) although the decline at low temperature was slower than that reported at ambient temperature as indicated by the complete disappearance of the bacterium within 16 days instead of 25 days at refrigerated temperature (Table 3).

Table 2: Survival of *E coli* inoculated into cultured buttermilk at different storage temperatures[a]

1st inoculum (~ 6 log CFU/ml)					2nd inoculum (2 log CFU/ml)			
5° C			25°C		5° C		25°C	
Time (Days)	pH	count[c]	pH [b]	count	pH	count	pH	count
0	4.3 ± 0.06	6.8 ± 0.1	_	_	4.4 ± 0.03	6.0 ± 0.3	_	_
3	4.4 ± 0.01	6.6 ± 0.4	4.4±0.01	4.3±1.0	4.4 ± 0.02	6.3± 0.2	4.3± 0.07	4.4± 0.8
5	4.5 ± 0.05	4.9 ± 0.0	4.3±0.03	3.5±0.7	4.5 ± 0.04	5.0± 0.1	4.3± 0.08	5.1± 0.3
12	4.4 ± 0.02	4.3 ± 0.0	4.3±.08	3.1±0.2	4.4 ± 0.02	4.8± 0.1	4.3± 0.07	2.6± 0.2
16	4.5 ± 0.05	2.9 ± 0.6	4.3±0.01	1.2±0.2	4.5 ± 0.04	3.2± 0.2	4.4± 0.07	<10
18	_	_	_[d]	<10				
25	4.4± 0.04	2.5 ± 0.2			4.2 ± 0.05	<10		
30	4.3 ± 0.06	<10						

[a] Post-heated milk samples were inoculated separately with two levels of *E.coli* just before addition of starter cultures and incubated at 21 °C for 20hr,then the final products were stored at 4 or 25°C

[b]All pH and *E.coli* counts are the mean of two samples each done in duplicate.

[c]count: log CFU/ml

Table3: survival of *E coli* inoculated into cultured buttermilk at different storage temperatures[a]

1st inoculum (6.1 log CFU/ml)					2nd inoculum (2.4 log CFU/ml)			
5°C			25°C		5°C		25 °C	
Time (Days)	pH	Count[c]	pH	count	pH	count	pH	count
0	4.3 ± 0.03	6.1± 2.3	4.3 ± 0.03	6.1± 2.3	4.13 ± 0.21	2.4 ± 0.3	4.1± 0.21	2.4 ± 0.3
1	4.4 ± 0.03	4.4 ± 0.1	4.3 ± 0.04	3.8 ± 0.2	4.43 ± 0.01	1.5 ± 0.0	4.3 ± 0.02	1.7± 0.1
3	4.4 ± 0.06	3.6 ± 0.0	4.3 ± 0.01	3.2 ± 0.1	4.37 ± 0.01	<10	4.2 ± 0.04	<10
16	4.4 ± 0.08	1.9 ± 0.1	4.3 ± 0.17	<10[b]				
18	--	1.6 ± 0.1						
21	4.5 ± 0.15	<10						

[a]Cultured buttermilk samples were prepared in the laboratory and the final products inoculated with *E.coli* bacterium and stored at different temperatures.

[b]Sampling was stopped when *E,coli* count was less than 10/ml.

[c]Count: Log CFU/ml

The same findings were reported by Mufandaedza *et al*., (2006) who found that the viable counts of *E.coli* significantly reduced (from 7 to 3log CFU/ml) when it was inoculated into milk previously fermented using *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* after 48hr. Also, Potashnik *et al*., (1972) reported that coliform bacteria completely disappeared within 24 hrs when the *Streptococcus lactis* based cultured milk product was stored at 37 or 43 °C whereas a slight decrease were found during storage at 5°C. Ogwaro *et al*., (2002) found that stationary phase *E. coli* O157:H7 inoculated post-fermentation survived during 4°C storage, but not 25°C storage. The data in (Table 3) show that no *E.coli* was recovered in the fermented milk inoculated with low inoculum's level (2.4 log CFU/ml) after

just 3 days of preservation at either 5 or 25^{o}C. Unlike the survival patterns observed when the bacterium was added before fermentation process it seems that the shock to the organism caused by the sudden exposure to the acid environment was the main cause of the fast dropping in the *E.coli* number when the bacterium was inoculated into previously fermented milk.

Survival of *S. typhimurium* in skimmed milk during and after fermentation

The long persistence of *E.coli* in fermented milk rises a big concern about the possibility of other human pathogens related to the family *Enterobacteriaceae* to survive and grow in the cultured buttermilk. Therefore another experiment was carried out in the same manner to determine to what extent *S.typhimuriun* can grow and survive in fermented milk as pre- or post fermentation contaminant. The experiment yielded the data shown in (Tables 4, 5, and 6). When initial inoculum sizes of 4.5 or 2.5 log CFU/ml was inoculated separately into the milk during fermentation to fermented milk *Salmonella* count increased during the first 6hrs of fermentation reaching a maximum count of 6.3 ± 0.1 and 4.1± 0.0 CFU/ml for two inoculum's sizes respectively and then started to decline thereafter (Table 4). These results were in agreement with the findings of Mufandaedza *et al*., (2006) who demonstrated that *S. enteritidis* survived and grew and reached high populations of about 8.8 log CFU/ml, after 18hrs when the bacterium was inoculated at the beginning of the fermentation. In this study during early stages of the fermentation changing in the *Salmonella* population coincided with a marked drop in pH values (4.0-4.2) for both tested inocula.

At the end of fermentation process and when the fermented milks were transferred to the refrigeration storage or kept at the ambient temperature again as reported in the *E.coli* experiment the pathogen survived longer at low temperature (5^{o}C±1) than at ambient temperature (25^{o}C ±2) (Table 5). However it seems that the survival of *S.typhimurium* in fermented milk during the refrigerated or ambient temperature storage is significantly different ($P< 0.05$) as the bacterium became undetectable at shorter time (within 3 or 5 days) for the high (4.5 log CFU/ml) and low (2.5 log CFU/ml) inoculum's size tested than that reported in *E.coli* experiment ((Table2). The same results were obtained by Park and Mark (1972b) who reported that *S. typhimurium* could be survived in fermented milk for 6 to 9 days at refrigerated temperature (11^{o}C). Again it was suggested that long survival of *S. typhimurium* in skimmed milk during fermentation and the subsequent refrigeration was returned to the reduction of the initial shock of acid environment to the cell and thus permit for long persistence of the bacterium. This was evidenced by the findings of Wen Shen *et.al*, (2007) who used acid adapted *S. typhimurium* (at pH 5.5 for 4 h) to investigate the viability during the lactic fermentation of skim milk with *Streptococcus thermophilus* or *Lactobacillus bulgaricus*, and during the storage of lactic fermented milk products. They demonstrated that acid adaptation, in addition to enhancing acid tolerance, reduced the susceptibility of *S. typhimurium* to refrigerated temperature and other detrimental factors which might be present in lactic fermented milk products.

In the present study when *S. typhimurium* was inoculated into previously fermented milk (as post-fermentation contaminant) again the fermented milk conditions did not allow the long survival of the pathogen as the bacterium was not detected after 2-3days of the storage at either 25°C or 5 °C for both high and low inoculun levels (Table 6). In this instance, fast dropping in *Salmonella* number may be attributed to the presence of lactic acid as reported by Park and Marth (1972b) where it is probably the single most important factor which causes demise of the *Salmonella*. In contrast, in another study when *S. typhimurium* was artificially inoculated into Egyptian yogurts and held at -1 to 4 °C or at room temperature (24 °C) the bacterium showed a long persistence in the products as it survived for 68 and 23 days at refrigeration and room temperature storage respectively (Ahmed and Ghoniemet , 1971). The differance in results obtained by Ahmed and Ghoniemet, (1971) and the results of the present study in terms of the short survival of *S. typhimuriun* (only 2or 3 days) compared with (68 and 23 days) in the previously mentioned study might be returned to the difference of the species of lactic acid bacteria used in each study. Park and Marth (1972a) indicated that survival (or inactivation) of *Salmonella* in cultured skimmed milks is related to the species of lactic acid bacterium used and the strain of such a species.

Table 4: Growth and survival of *S.typhimurium* in milk during fermentation to cultured buttermilk [a]

1st inoculum (~ 5 log CFU/ml)			2nd inoculum (~ 3 log CFU/ml)	
Time (Hours)	pH	count[b]	pH	count
0	6.62 ± 0.0	4.7 ± 0.2	6.8 ± 0.10	2.8 ± 0.1
6	5.2 ± 0.04	6.3 ± 0.1	5.4 ± 0.0	4.1± 0.0
12	4.3 ± 0.1	5.8 ± 0.40	4.4 ± 0.03	3.2 ± 0.2
20	4.0 ± 0.07	3.9 ± 0.42	4.2 ± 0.01	2.2 ± 0.3

[a] Post-heated milk samples were inoculated with two levels of *S. typhimurium* just before addition of starter cultures and incubated at 21°C for 20hr,then the final products were stored at 4 or 25°C

[b]All pH values and *Salmonella* counts are the mean of two samples each done in duplicate. [c]count: Log CFU/ml

Table 5: Survival of *S.typhimurium* in the cultured buttermilk during storage at either 5 or 25 °C

1st inoculum (~ 5 log CFU/ml)					2nd inoculum (~ 3 log CFU/ml)			
Storage temperature[a]					Storage temperature			
	5 °C		25 °C		5 °C		25 °C	
Time (Days)	pH[b]	Count[c]	pH	count	pH	count	pH	count
0	4.± 0.07	3.9 ±0.4	4.0. ± 07	3.9 ± 0.4	4.0 ± 0.07	2.2 ± 0.3	4 ± 0.07	2.2 ± 0.3
2	4.2± 0.04	3.8 ± 0.1	4.1± 0.04	2.2 ± 0.2	4.3 ± 0.01	2.3 ± 0.0	4.0 ± 0.1	1.9 ± 0.2
3	4.3 ± 0.1	2.2 ± 0.1	4.3 ± 0.01	< 10	4.4 ± 0.03	1.7 ± 0.1	4.3 ± 0.02	<10
5	4.2 ± 0.1	<10	_[d]		4.4 ± 0.02	<10		

[a] Post-heated milk samples were inoculated with two levels of *S. typhimurium* just before addition of starter cultures and incubated at 21°C for 20hr,then the final products were stored at 4 or 25°C

[b]All pH and *Salmonella* counts are the mean of two samples each done in duplicate.

[c]count: Log CFU/ml

[d] Sampling was stopped when *Salmonella* counts ware less than 10/ml.

Table 6: Survival of *S.typhimurium* inoculated into cultured buttermilk at different storage temperatures [a]

1st inoculum (4.5 log CFU/ml)					2nd inoculum (~ 2.5 log CFU/ml)			
5°C			25°C		5°C		25°C	
time (hr)	pH	Count[b]	pH	count	pH	count	pH	count
0	4.1± 0.1	4.5 ± 0.3	4.1± 0.1	4.5 ±0.3	4.21 ±0.11	2.5 ± 0.25	4.21 ±0.11	2.5 ± 0.25
24	4.2 ± 0.1	0.7 ± 0.41	4.2 ± 0.01	1.2 ± 0.04	4.31 ± 0.01	0.79 ± 0.3	4.19 ±0.13	0.8 ± 0.14
48	4.3 ± 0.1	1.0 ±1.0	4.1 ± 0.10	< 10	4.22 ± 0.05	<10	4.21± 0.03	<10
72	4.4 ± 0.01	<10[c]						

[a]**cultured buttermilks were prepared in the laboratory and the final products were inoculated separately with two levels of the bacterium and stored at 4C or 25 °C.**
[b]**All pH and *Salmonella* counts are the mean of two samples each done in duplicate.**
[c]**Sampling was stopped when *E,coli* count was less than 10/ml.**

In this study, generally survival patterns of *S.typhimurium* was different from that of *E.coli* as the later is more resistant and tends to persist longer in the product than *S.typhimuriun* and this may be returned to the possible presence of antibiotics produced by some lactics (Kulshrestha and Marth, 1974) or production of volatile compounds inhibitory to the bacterial growth (Marth, 1966). It was clearly evidence that the starter cultures used in the manufacture of cultured buttermilk do not guarantee the safety or quality of that product if post–heat treatment contamination occurred as evidenced by growth of *E.coli* and *S.typhimurium* in milk during fermentation and the long persistence of them in subsequence refrigerated milks.

Although the survival factors of *E.coli* or *Salmonella* in refrigerated fermented dairy products was previously stated by several workers (Goel *et al*., 1971; Park and Marth, 1972b; Potashnik *et al*., 1972; Park and Mark, 1973) the results of the present study confirm them and extend factors to include the stage of bacterial contamination (pre-or post fermentation) as one of the main factor to enhance or suppress the survival of both bacteria tested. Therefore contamination of heated milk should be avoided to maintain safety and quality of these products.

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نمـــو وبقائيـــة بكتريـــا *Escherichia coli* و *Salmonella typhimurium* في لبن الخض المتخمر التجارى

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أجريت هذه الدراسة لمعرفة تأثير كل من مستوى التلقيح , مرحلة التلقيح (قبل أو بعد عمليـــة التخمـــر) و درجـــة حـــرارة التخـــزين علـــى نمـــو وبقائيـــة بكتيريـــا *E.coli* و *S.typhimurium* في لبن الخض المتخمر التجاري. عندما تم تلقيح الحليب بمستويي تلقيح (2 or 6 logCFU/ml) من بكتيريا *E.coli* قبل عملية التخمر زادت إعداد هذه البكتيريا إثناء التخمر إلى٣ و ١,٥ أضعاف و ذللك لمستويي التلقيح بعد ٦, ١٢ساعه من عملية التخمر على التوالي. انخفضت الأعداد بعد ذلك تدريجيا عندما خزن اللبن المتخمر على درجة حرارة ٥ او ٢٥° م على الرغم من أن الانخفاض على درجة ٢٥ كان أسرع لتختفي البكتيريا بعد ٣٠ و ١٨ يوم لدرجتي الحرارة المختبرة على التوالي و ذلك لمستوى التلقيح العالي. في المقابل اختفت البكتيريا بعد ١٦ و ٢٥ يوم من التخزين على ٢٥ و ٥° م على التوالى فى اللبن ذى مستوى التلقيح الأقل.

عندما حقنت بكتيريا *E. coli* بمستويي تلقيح (2.6 or 6.1 log CFU/ml) مباشرة في الحليب المتخمر وبعد التخزين تحت نفس درجات الحرارة (٢٥ ,٥° م) لوحظ انخفاض مستمر في إعداد البكتيريا واختفت بشكل اسرع على درجة حرارة ٢٥ (١٦ يوم) عنها فى حالة اللبن المتخمر المخزن على٥° م (٢١ يوم) وذلك لمستوى التلقيح العالي بينما اختفاء البكتيريا كان الأسرع (٣ ايام) عند مستوى التلقيح الأقل.

أظهرت بكتيريا *S.typhimurium* نفس سلوك بكتيريا *E.coli* *تقريبا* عندما حقنت في الحليب قبل عملية التخمر بمستويي تلقيح هما(2.5 or 4.5 log CFU/ml) على الرغم من إن بقائيتها في اللبن كانت اقل من *E.coli* فى العموم . اقل بقائية لبكتيريا *S.typhimurium* كانت عندما حقنت في المنتج المتخمر مباشرة بعد التخمر حيث اختفت بعد أيام قليلة (٢ أو ٣ أيام) في المنتج المخزن على٢٥° و٥° م على التوالي.

في ضوء نتائج هذه الدراسة أتضح أن سلامة وجودة اللبن المتخمر التجاري مرتبطة بمرحلة تلوث اللبن (قبل او بعد عملية التخمر) و كذلك بدرجة حرارة تخزين المنتج النهائي.