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BEHAVIOR OF ENTEROHEMORRHAGIC ESCHERICHIA COLI AND ENTEROTOXIGENIC STAPHYLOCOCCUS AUREUS IN YOGHURT AND ACIDIFIED MILK

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SUMMARY

The present experiment was carried out to study the effect of processing of yoghurt, and cold storage of both yoghurt and acidified milk on the survival and growth of two reference strains. *Escherichia coli* O157: H7 and Enterotoxigenic *Staphylococcus aureus* were inoculated into milk in low (10^3CFU/ml) and high levels (10^6CFU/ml). The obtained results revealed that *E. coli* O157: H7 showed slight increase in viable cell count either at low or high concentration, while *S. aureus* showed gradual decrease in both low and high concentrations during processing of yoghurt. On the other hand the organisms showed another pattern of growth at storage temperature. In stored yoghurt at 4°C, *E. coli* O157: H7 decreased gradually from 4.23 to 1.95 log CFU/g and by about 3 log phase after 7 days of storage, then the organism was completely disappeared after 10 and 25 days for low and high dose respectively. While in acidified milk the viable cell count of *E. coli* decreased from 4.36 to 2.90 log CFU/g after 25 days of cold storage. *S. aureus* viable cell count was sharply decreased in stored yoghurt from 4.34 to 2.04 log CFU/g and from 7.17 to 2.11 log CFU/g after 3 and 10 days at low and high concentration respectively, while in acidified milk the organism showed a decrease in count from 4.39 to 1.69 log CFU/g after 15 days of cold storage. Changes in the pH of the examined products were studied. The public health importance of the tested organisms and suggested control measures to improve keeping quality of yoghurt were discussed.

INTRODUCTION

Lactic acid bacteria and their fermented food products are thought to confer a variety of impor-

tant nutritional and therapeutic benefits to consumers, including antimutagenic and anticarcinogenic activity (Fernandes et al., 1987 and Gilliland, 1990). Epidemiological evidence indicates a negative correlation between the incidence of certain cancers and consumption of fermented milk products (Bueno de Mesquita et al., 1991, Van't Veer et al., 1991 and Peters et al., 1992).

Yoghurt is a popular fermented dairy product as it is consumed by different ages for its good taste and high digestibility. The product may be subjected to contamination by different pathogens as *E. coli* O157: H7 and *S. aureus* which may get access into it either before, during or even after processing, rendering the product unsafe for consumption.

Enterohemorrhagic *E. coli* O157: H7 is recognized as an important and common human pathogen, particularly foryoung children and the elderly, causing diarrhoea, haemorrhagic colitis and the life-threatening post-diarrhoeal disorders of haemolytic uraemic syndrome (HUS), (Riley et al., 1983; Karmali, 1989; Morgan et al., 1993 and Tarr, 1994). Staphylococcal food poisoning is a syndrome characterized by gastrointestinal disturbance, although the disease is characterized by low mortality rate and short duration, the frequency of out-breaks and severity of the symptoms mark staphylococcal food poisoning as an important foodborne hazard in many kinds of food stuffs (Adrian, 1992). Survival of foodborne pathogens in acidic environment is influenced by a number of environmental and cultural factors. Although metabolites produced by starter culture could inhibit them (Motlagh et al., 1991).

Yet, factors may interfere with acid fermentation can free the way for these organisms to grow and become hazardous for public health. Therefore, this work was planned to monitor the effect of yoghurt processing and storage of both yoghurt and acidified milk at 4°C on the survival of these organisms.

MATERIAL AND METHODS

Cultures:

1- Strains used in this study were kindly provided by the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany.
2- Starter culture was obtained from Hansen's Laboratorium, Denmark.

Preparation of inoculum:

E. coli O157: H7 and *S. aureus* strains were transferred from refrigerated stock agar slant culture to sterile Tryptic Soya broth containing 0.6% yeast extract (TSBY) and incubated at 35°C for 18 to 24 hours. After two successive transfers and incubations, the cultures confirmed to be pure on specific media before inoculated into samples (ATCC, 1992). The working starter culture was prepared in sterile skimmed milk 24 hours before

yoghurt processing.

Processing of yoghurt:

Milk was heated for 10 minutes at 90°C, then cold to 42°C and inoculated with starter culture (2% v/v). Inoculated bulk milk was divided into five parts (500 ml each). Two of them were inoculated with one of the prepared pathogen (*E. coli*) culture in TSBY broth to obtain final count of 10^3 and 10^6 CFU/ml, the other two parts were inoculated with the second pathogen (*S. aureus*) culture to obtain the same count. The fifth part was left as a control for the different experiment steps.

Preparation of acidified milk:

Milk was heated as mentioned before and cold at room temperature then adjusted the pH (4.77) at the beginning of storage at 4°C of the control yoghurt by using lactic acid. Acidified milk was divided into three portions (500 ml each). The first was inoculated with TSBY broth to obtain final count of 10^3 CFU/ml of *E. coli* and the second part was inoculated with the same count of *S. aureus*, the third part was left as a control.

Design of the experiment:

Three trials plan was designed to assess the effect of yoghurt processing and storage temperature of yoghurt and acidified milk on the growth and survival of *E. coli* O157:H7 and *S. aureus*. The different portions of milk were tested for viable cell count of *E. coli* and *S. aureus* at zero and fifth hour of yoghurt processing then after storage at 4°C of yoghurt and acidified milk for zero time first day, 2nd day, 3rd day, 5th day, 7th day, 10th day, 15th day, 20th day and 25th day.

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

Count was proceeded after preparation of samples according to (APHA, 1985) and surface plating onto Sorbitol MacConkey Agar and Baird Parker Agar (APHA, 1992) before incubation of plates at 35°C for 24 and 48 hours respectively. Samples from the control portion were tested along the experiment.

Determination of pH:

The pH was measured at the time of testing, by direct inserting the electrode (pH meter SUNTEX TS-1, Electrode INGOLD U455/120/M) into well-mixed samples.

RESULTS and DISCUSSION

Acid resistance and acid tolerance are important virulence determinants that contribute to the survival and pathogenicity of infectious foodborne pathogens to cause disease. Acid resistance increases the portion of the population that survive the gastric environment and further appears to increase infectivity once the pathogen attaches to the intestinal tract (Peterson, et al., 1989 and Gordon and Small, 1993). In addition, induction of acid resistance or acid tolerance can increase bacterial resistance to other stresses such as heat,

ionizing and non-ionizing irradiation, and antimicrobial agents (Goodson and Rowbury, 1991 and Buchanan, et al., 1999).

The achieved results from this study revealed that *E. coli* O157: H7 was increased in count from 4.14 and 7.60 to 4.23 and 8.04 log CFU/ml and / or g. for both low and high level of inoculum respectively, owing to the effect of the processing, while *S. aureus* was slightly decreased from 4.38 and 7.20 to 4.34 and 7.17 log CFU/ ml and / or g. for low and high count respectively. pH was reduced from 6.18 to 4.77 (Table, 1).

Table (2) & Fig. (1,2) indicates that the count of *E. coli* O157: H7 was reduced by one log phase in the 2nd and 7th day of storage before completely failed to be detected after 10th day. Results declared that, using low inoculum (low level of contamination) of the organism could be eliminated after 10 days of storage at 4°C. While the high inoculum (highly contaminated product) may persist for longer time extending to 20 days and diminishing by low rate (one log phase). Al-Ashmawy et al. (1989) and Hudson et al. (1997) reported nearly similar findings. While El-Hawary and Aman (1998) reported that *E. coli* O157: H7 count (2.3 x 10^5 CFU/g) was diminished to zero after 11 days. The low inoculum of *S. aureus* reduced by one log phase rate in the first day of storage before completely disappeared after 5th day. While in high inoculum *S. aureus* was reduced by 3-log phase after 3 days, its count was diminished to 2.11 log CFU/g at 10th day and

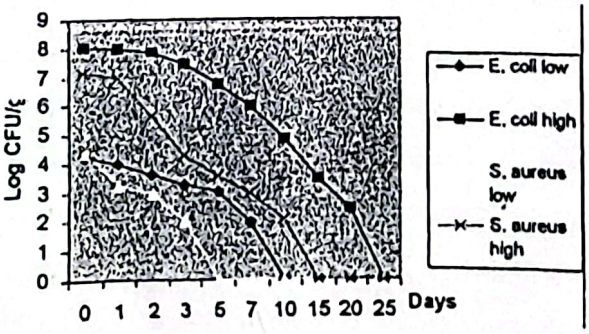

Fig. 1: Effect of storage temperatures on viability of *E. coli* and *S. aureus* in yoghurt.

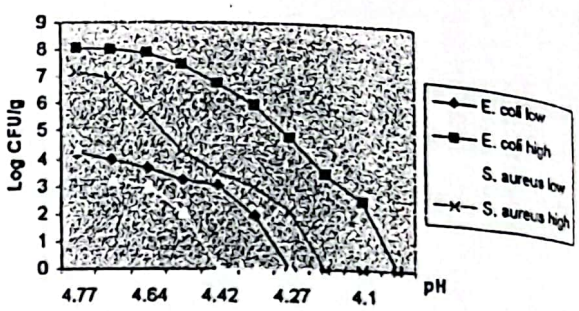

Fig. 2: Effect of pH on viability of *E. coli* and *S. saureus* in yoghurt.

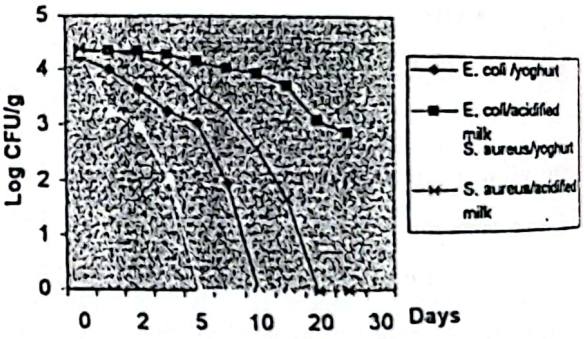

Fig. 3: Effect of storage temperatures on *E. coli* and *S. aureus* in yoghurt and acidified milk

failed to be detected after this period. Nearly similar agreement was reported by Al-Ashmawy et al. (1989). While Abd El-Hady, (1998) reported that *S. aureus* count (6.7 log CFU/g) diminished to zero after 18 days.

Results presented in Table (3) & Fig. (3) revealed that the pathogenic organisms lost its viability in acidified milk gradually during storage at 4°C, at which the pH was slightly decreased. *E. coli* O157:H7 proved to be more resistant and could be isolated from acidified milk up to 25 days

Table (1): Effect of yoghurt processing on survival of *E. coli* O157:H7 and *S. aureus*.

Processing time	pH	Log CFU/ ml.g			
		E. coli O157:H7		S. aureus	
		Low	High	Low	High
0	6.18	4.14	7.60	4.38	7.20
5 hours	4.77	4.23	8.04	4.34	7.17

Table (2): Growth behavior of *E. coli* O157:H7 and *S. aureus* in yoghurt stored at 4°C.

Storage time/day	pH	Log CFU/ ml.g			
		E. coli O157:H7		*S. aureus*	
		Low	High	Low	High
0	4.77	4.23	8.04	4.34	7.17
1	4.70	4.00	8.00	3.25	6.97
2	4.64	3.65	7.89	3.00	5.67
3	4.51	3.25	7.47	2.04	4.30
5	4.42	3.04	6.74	0.00	3.55
7	4.35	1.95	5.97	0.00	3.00
10	4.27	0.00	4.81	0.0	2.11
15	4.18	0.00	3.46	0.00	0.00
20	4.10	0.00	2.47	0.00	0.00
25	4.00	0.00	0.00	0.00	0.00

Table (3) Growth behavior of *E. coli* O157:H7 and *S. aureus* in acidified milk stored at 4°C.

Storage time/day	pH	Log CFU/ ml.g	
		E. coli O157:H7	*S. aureus*
0	4.77	4.36	4.39
1	4.76	4.36	4.34
2	4.75	4.34	4.25
3	4.73	4.30	4.07
5	4.72	4.17	3.63
7	4.70	4.04	3.27
10	4.69	3.95	2.60
15	4.67	3.73	1.69
20	4.66	3.11	0.00
25	4.64	2.90	0.00

Table (4) Correlation coefficient between the examined parameters

	Correlation coefficient	Coefficient of determination (R^2) %
Yoghurt	-	-
Days Vs	-	-
pH	- 0.95	90.25
E. coli O157:H7low dose	- 0.90	81.00
E. coli O157:H7high dose	- 0.99	98.01
S. aureus low dose	- 0.72	51.84
S. aureus high dose	- 0.92	84.64
pH Vs	-	-
E. coli O157:H7low dose	0.95	90.25
E. coli O157:H7high dose	0.95	90.25
S. aureus low dose	0.89	79.21
S. aureus high dose	0.98	96.04
Acidified milk	-	-
Days Vs	-	-
pH	- 0.96	92.16
E. coli O157:H7	- 0.99	98.01
S. aureus	- 0.99	98.01
pH Vs	-	-
E. coli O157:H7	0.91	82.81
S. aureus	0.94	88.36

storage at 4°C, while *S. aureus* still survived for 15 days at the same storage temperature. Comparing the ability of the microorganism for surviving in stored yoghurt and acidified milk, it could be concluded that *E. coli* O157:H7 had been survived in yoghurt up to 7 days at starting level 4.23 log CFU /g inoculum and in acidified milk up to 25 days at starting level 4.36 log CFU/ml. While *S. aureus* completely disappeared after 3 days and 15 days in yoghurt and acidified milk at nearly the same level of inoculum respectively. It's commonly known that *E. coli* has the ability to resist and tolerate high acidic media more than *S. aureus*. While the two organisms showed high persistence in acidified milk more than in yoghurt due to the effect of starter culture metabolites (Kabara et al., 1972; Freese et al., 1973; Kabara, 1981 and Schaffer et al., 1995).

It is declared from these results that the surviving period of the inoculated food poisoning organisms in yoghurt and acidified milk depends on the dose of inoculum, storage time and the rate of the developed acidity of the products (Table 4).

In conclusion, adequate hygienic measures during processing, distribution of yoghurt should be adopted, by controlling the storage temperature, maintaining pH, observing purity of starter cultures and preventing post-processing contamination to safeguard consumers.

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