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SURVIVABILITY OF *ESCHERICHIA COLI* 0157:H7 AND NON-0157 SEROTYPES ISOLATED FROM SOME DAIRY PRODUCTS UNDER STRESS CONDITIONS

[42]

Zeinab I. Sadek[1]

ABSTRACT

Microbiological assay of 150 samples of dairy products for the incidence of Coliforms, *E. coli*, *E. coli* 0157:H7 and non-0157 *E.coli*, show the recovery of the Coliforms and *E.coli* from the tested products with different incidence percentage. Strains of *E. coli* 0157:H7 and non-0157 *E .coli* were recovered from 9 (6%) and 13(8.6%) of the total samples, respectively. The behaviour of these isolates were tested when exposing to low pH, salt, low holding temperature and heat treatment. The results indicate obvious survival of *E. coli* 0157:H7 and non-0157serotype with pH as low as 3.8 pH for 5 days. Also, survival or even multiplication in TSB containing <6% salt. Moreover, the strains remained viable at low holding temperature (5^{o}C). So, the product which contaminated with this pathogens remain hazardous even under such stress condition. Decimal reduction times (D-values) of cells suspended in saline solution, TSB medium, reconctituted dry milk and chocolate milk were determined. The greatest survival as evidenced by highest D and Z values occurred with chocolate milk. Product composition and type of strain affected the heat lethality rates.

Keywords: *Escherichia coli* 0157:H7, non-0157 *E. coli*, pH, Salt, Heat resistance, Dairy products

INTRODUCTION

Most of *Escherichia coli* different strains are of special interest for its potential health hazard, as serotypes causing diarrhea or more serious forms of illness.

Enterohaemorrhagic *E. coli* (EHEC) is the most pathogenic strain among the verotoxin (VTEC) or shiga toxin producing *E. coli*. The illness caused by VTEC can range from self-limited, watery and bloody diarrhaea to life threatening manifestations such as haemolytic uraemic syndrome (HUS) or thrombotic thrombocytopenic purpura, which may result in human death **(Padhye and Doyle, 1992)**. Although, a wide variety of VTEC serogroups has been implicated in human disease *E. coli* 0157:H7 is the most prevalent strain.

Also, serotypes other than 0157:H7 (non-0157) such as 026, 0103, 0111and o145 have been identified as emergent

1- Dairy Department, National Research Centre, Dokki, Cairo, Egypt.

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human pathogen **(McKee *et al* 2003).** Several outbreaks were attributabe to serotypes 026:H11, 0111:H8 and 0121:H19 **(Large *et al* 2005).** Non- 0157 *E. coli* were also thought to have a low infections dose as *E. coli* 0157:H7 **(Large *et al* 2005).**

Survival of *E .coli* 0157:H7 already has been demonstrated in Cottage, Cheddar, Colby, Romano, Feta and soft cheeses, skimmlk; buttermilk; sourcream and yogurt **(Arocha *et al* 1992; Guraya *et al* 1998; Reitsma and Henning 1996 and Ogwaro *et al* 2002)**. Also, non-157 strains have been isolated from raw milk, ice cream, yoghurt, kareish, cheese, Domiati cheese and Ras cheese **(El-Ashmawy *et al* 2005 and Mckee *et al* 2003).**

Acidity is an important parameter influencing the ability of pathogen to cause disease. Since its resistance to high acid and low pHs may permit the pathogen to survive in acid foods and acidic enviroment of the stomach.

The common use of NaCl to salten foods also creates attendance to suppress the potential pathogens by elevating NaCl concentrations.

Keeping low -temperature of foods is still the primary mean utilized to restrict the growth of bacteria and pathogens in foods .However, a group of pathogens were capable of growing at 5^{o}C including *E. coli* **(Palumbo *et al* 1997).** *E. coli* 0157:H7 has been shown to survive in acidic foods stored at refrigeration temperature better than those at ambient temperature **(Zhao *et al* 1993).**

Providing a successful heat-treatment is a critical point for controlling *E. coli* in many manufactured foods. Data on heat-resistance are therefore, essential to obtain a safe heat-treatment especially as refrigeration cannot be relied upon to prevent growth of this pathogen.

Therefore, the objective of this study is to detect the prevalence rates of *E. coli* 0157:H7 and non-157 *E. coli* strains in milk and some dairy products to assess their susceptibility to various environmental and food-related process factors such as acidity, salinity, keeping at low temperature and heat treatment.

This will be of great benefit for milk and milk products process, hygiene and keeping quality.

MATERIAL AND METHODS

Samples collection

Samples were collected from different dairy farms, super markets, retailar shops over one year period. One hundred and fifty samples (30 each of raw milk, butter, Domiati cheese, kareish cheese and ice cream)were delivered directly to the laboratory as required by American Public Health Association **(A.P.H.A., 1992).**

Eneumeration of Coliforms and *E.coli* and isolation of *E. coli* 0157:H7 and non-157 *E. coli*

Samples were enriched in MacConkey broth medium **(El-Nokrashy *et al* 1992)** and examined for incidence of total colifors in a set of 3 fermentation tube of MacConkey broth with inverted Durham's tube for collection of gas and incubated at 37^{o}C for 48h. according to and a loopful from the positive tubes was streaked on levine's Eosin Methylene Blue agar and incubated at 44^{o}C for 24h.and examied for typical colonies (metalhic-shiney green) of *E.coli*.Also,0.1 ml was spread onto dried surface of Ce-

fixime Tellurite Sorbitol MacConkey agar (CT.SMAC) (Oxiod),plates were incubated at 37°c for 24 hrs**, (Zadik *et al* 1993)** colourless colories were picked up and steaked onto Tryptone soya agar slants for identification of *E.coli* 0157:H7 and non-157 serogroups.

Identification methods

Identification methods were carried out according to **Kreig and Holt (1991).** The immunological detection system was used, which include *E. coli* 0157 latex agglutination test (Oxoid)and latex stide agglutination test for the detection of non-0157 serogroups (026, 091, 0103, 0111, 0128 and 0145) (Dry spot *E. coli* seroscreen (Oxoid).

Preparation of inculum

Isolates of *E .coli* 0157:H7 and non-157 *E. coli* were grown separately in TSB and incubaled at 37°C,equal volumes of 18h cultures of the isolated strain from the same source of dairy products were combined to serve as the inoculum for each experiment.

pH tolerance

Tryptone soy broth (TSB) was adjusted aseptically to pH values (3.8, 4.1, 4.7, 5 and 5.5) with strile 85% lactic acid.TSB in different pHs were inoculated with 10^5 CFU/ml, kept at 10°C and analyzed after 2 and 5 days onto Violet Red Bile Agar (VRBA) (Oxoid). The experiment was carried out in doublicate.

NaCl tolerance

The experimental was similar to the pH study, but TSB medium was adjusted to pH 7 and each containing amounts of NaCl to yield 0,2,4,6and 8%.

Minimum growth temperature

The minimum growth temperature was determined by testing the isolates of *E.coli* 0157:H7 and non-157 serogroup in which grown as described before in TSB at temperature 10,8 and 5°C for 10 days at intervals of 0,3,5,7and 10 days. Aliquots of *E. coli* strains were determined for viable cell counts by plating on VRBA for determination of maximum population at the minimum temperature.

Heat Resistance

a- Strain selection

Isolates of *E. coli* 0157:H7 and non-157 serogroups were examined for their heat resistance in TSB.

Preliminary experiment were done by exposing all isolates (24h-old) to different heat treatments (73°C/15 sec. and 60°C/ 30 min.). The preferable heat treatment to determine the heat-resistance parameters for the isolates was 60°C 10 min. The most resistant 2 strains of each of *E. coli* 0157:H7 and non-157 serogroups were chosen to determine D and Z-values.

Using the test tube method **(Donnelly and Briggs, 1987),** the two strains (24h-old) were inoculated (0.1ml) into screw-capped test tubes containing 10 ml of sterilized saline solution, TSB, reconstituted milk or chocolate milk, and heated at 55, 60 and 65°C in a water bath began at the end of the come-up period, with dwell times at each temperature. Time/ Temperature treatments were carried out as follow:

55°C:10, 20, 30, 40and 50 min.

60°C:0.5, 1, 2, 3, 4, 5, 6 and 10 min.

65°C:20, 60, 80, 120, 180, 200 and 240sec.

b- D and Z- value catculations

Rates of thermal inactivation of each bacterium of *E. coli* 0157:H7 and non-157 serotypes were determined graphically by plotting the $\log_{10}$ cfu/ml of surviving cell population (detecting by using plate count agar) versus heating time for each temperature. The best fit line was drawn through the date points, and D-values were obtained from the slope of the best fit lines.Z-value of each bacterium was estimated by regressing log D-values versus heating temperatures **(Ahmed *et al* 1995).**

RESULT AND DISCUSSION

Incidence of VTEC0157 and non-0157 VTEC in dairy products

Results of the 150 tested samples of dairy products are listed in **Table (1)** for the presence of Coliforms,

E. coli, verocytotoxigenic *E. coli* (VTEC) 0157:H7 and non-0157 serotypes. Results indicated the high levels of contamination with Coliforms (100%) in raw milk samples, which consequently indicates a probable sanitation problemes in farms, marketing and handling of raw milk.

Table 1. Incidence of coliforms and *E. coli*, VTEC-0157 and Non-0157 VTEC isolates in milk and some milk products.

Tested samples	Total No. of sample	Total coliforms		*E. coli*		VTEC-0157		Non-0157 VTEC	
		No. of positive samples	%	No. of positive samples	%	No. of positive samples	%	No. of positive samples	%
Raw milk	30	30	100	24	80	3	10	2	6.6
Butter	30	13	43.3	5	16.6	0	0	0	0
Domiati cheese	30	20	66.6	8	26.6	2	6.6	2	6.6
Kareish cheese	30	25	83.3	19	63.3	3	10	6	20
Ice cream	30	9	30	6	20	1	3.3	3	6.6
Total	150	97	64.6	62	41.3	9	6	13	8.6

Total No. of . *E. coli* 0157:H7 isolates = 14

Total No. of . non –*E. coli* 0157:H7 isolates = 20

Also, raw milk samples showed high incidence of VTEC 0157 (10%) and non-157 VTEC (6.6%). Similar results were obtained by **Abdul-Roouf *et al* (1996)** who reported that 6% of raw cows' milk samples examined in Egypt were contaminated with *E. coli* 0157:H7. Moreover, **Massa *et al* (1999)** recorded a good survival or even multiplication of *E. coli* 0157:H7 in raw milk when stored at 8°C.

Although these organisms would not be expected to survive pasteurization of raw milk the cheeses made from this milk might represent a health hazard if the pathogen was there and survived the cheese making process, Hereupon the prevalence rates of coliforms, *E. coli* 0157:H7 and non 0157 type were high in Kareish cheese (83, 10 and 20%, respectively). While in Domiati cheese the incidence percent was 66.6% coliforms and 6.6% for each of VTEC 0157 and non-0157 types. Known enterotoxigenic stains have been shown to survive during manufacture of brick and Camembert cheeses **(Glatz and Brudvig, 1980)**, Cheddar Cheese **(Reitsma and Hening, 1996)**, Domiati and Kareish **(Ahmed *et al* 1988 and Abd-El-Hady *et al* 1995)** and Cottage cheese **(Arocha *et al* 1992)**. Moreover, **Hussein and Sakuma (2005)** reported that *E. coli* 0157:H7 not only survived the manufacturing process of Comembert and feta cheeses but increased in number at the end of storage period.

E. coli 0157:H7 and non-157 serogroups failed be to detected in butter samples **(Table 1).**

This was may be due to physical characteristics and low water activity in this product limits survival and growth of *E. coli*. The minimum water activity for growth of *Eschericha* species is 0.95 **(Glatz and Brudvig, 1980)** well above the water activity of butter.

Ice cream samples **(Table 1)** had lowest percentage of Coliforms and only one of the 30 ice cream samples (3.3%) contained VTEC-0157 but three samples (10%) were positive for non-0157 VTEC. The non-0157 VTEC are more frequently present in food of animal origin than serotype 0157 **(Neill, 1997). El-Ashmawy *et al* (2005)** could isolate VTEC 0157:H7 and non-0157 from ice cream samples in lower percentages (2%for each strain).

Acidity tolerance

The survival and growth respones of isolates of *E. coli* 157:H7 to different pHs (3.8-5.5) were illustrated in **Figs. (1 & 2),** as indicator for acidity tolerance. At pH 3.8, the isolated strains of *E.coli* 0157:H7 toleraled such a low pH in 2 days incubation and survived but with sharp rate of decline at the 5th day of incubation.

The bacteria count at pH4.1 exhibited ~2-log reduction after 5 days of incubation of the isolated strains from different tested dairy products. Survival with no growth was observed at PH 4.4 after 2 and 5 days of incubation of all of the tested strains of *E. coli* 0157:H7. Therefore; the results indicated that the tested strains may grow and increase in counts between pH 5 and 5.5 as low acid to neutral medium. While, these were decline in number below pH 5 in the high acid medium across 2-days. Difference in cell densities at different pH level were observed between the strains isolated from different samples of tested dairy products. In this respect**, Zhao *et al* (1993)** reported that this pathogen grew slightly in apple cider at pH 3.8 to 4, indicating more acid

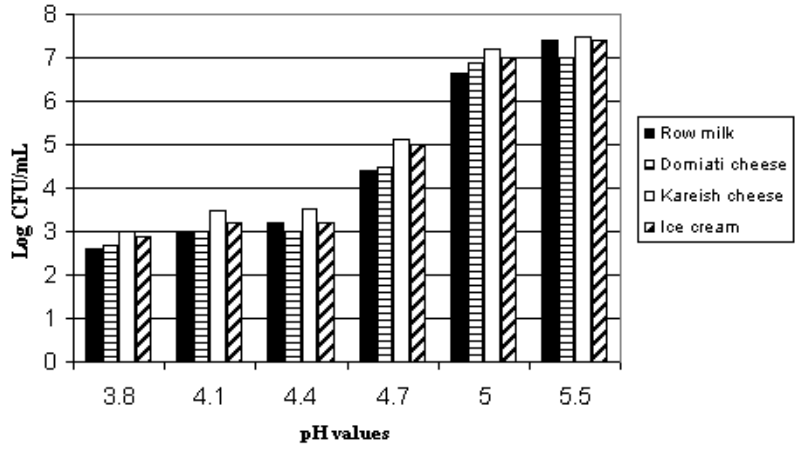

Fig. 1. Growth response of isolated strains of *E. coli* 0157:H7 in TBS at varying pH levels at 10°C for 48 h.

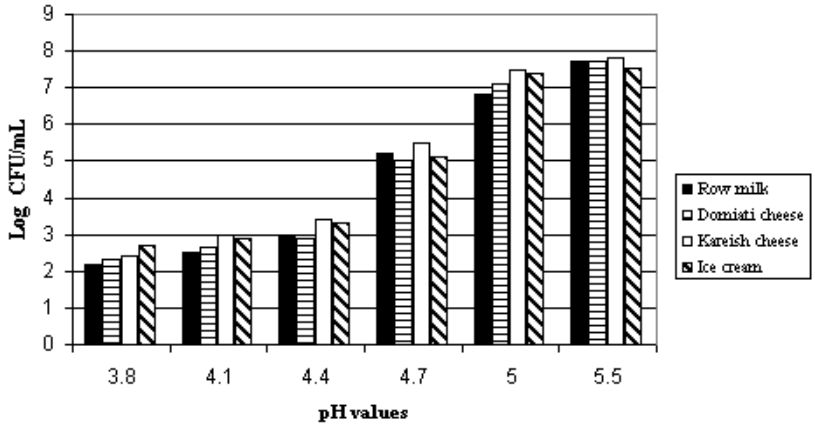

Fig. 2. Growth response of isolated strains of *E. coli* 0157:H7 in TBS at varying pH levels at 10°C for 48 h.

tolerance than observed in this study. Also, **Besser *et al* (1993)** noticed survival of *E. coli* 0157:H7 at 8^{o}C for 20 days in freshly pressed apple cider with pH below 4.Moreover, **Guraya *et al* (1998)** found that *E. coli* 0157:H7 was survived at pH 3.8 in skin milk for 7 days and was not detected at pH 4.4 after 35 days and in butter milk, sour cream, and yogurt, *E. coli* 0157:H7 was inactivated at rates similar to or greater than those for skimmilk. Moreover**, Hussein and Sakuma (2005)** reported that *E.coli* 0157:H7 survived in yogurt and sour cream at low pH values (4.17and 4.3, respectively). In contrarly, **Guraya *et al* (1998)** reported that *E. coli* 0157:H7 did not survive in skim milk at pH 3.8 and was reduced by 3-log cycles at pH 4.1.

On the other hand, strins of *E. coli* 0157:H7 isolated from Kariesh cheese achieved higher densities compared with the other studied strains for the both 2 and 5-day incubation period in all pH-values.

The survival and or growth of isolated *E. coli* non157in TBS adjusted to various pH values for 2 and 5-days incubation at 10^{0}C are shown in **Figs. (3 & 4).**

There is a decrease in the viable courts of all of the isolated strains at pH values 4 and 3.8. While, viable courts increased in TBS at pHs 5.5 and 5, whereas, the organism grew up to ~2-log cycles at pH5 after 2-days incubation and more after 5-days.Slight differences were observed in rates of inactivation, survival and growth at different pH values between the strains isolated from different samples of diary products. In general, strains of *E. coli* 0157:H7 vary widely in their acid tolerance **(Miller and Kasper, 1994 and Massa *et al* 1997).**

It should be pointed out that the isolated strain of non-0157 *E. coli* have less ability to survive in acidic media than isolated strains of *E. coli* 0157:H7. In this respect, **Large *et al* (2005)** reported out breaks associated with non-157 *E. coli* was much less frequently than *E. coli* 0157:H7 and the reason for this disparity in prevalence may result from the differences in the inherent acid resistance and survival in acidic food between the two types. Also, **Eblen *et al* (2005)** reported that strains other than *E. coli* 0157:H7 do not have the high acid resistance reported for *E.coli* 0157:H7.

Generally, acid survival plays an important role in bacterial enteric infections. Food borne pathogens must survive in the stomach (pH<3). The data indicate that products at or below pH 4 could be good vehicles of *E. coli* 0157:H7 and non 157 strains.

Salt tolerance

In the presence of NaCl, the range of growth of *E. coli* 0157:H7 was between 2-4% NaCl, and this rate was decline with 6% NaCl during 2 and 5-days incubation in TSB at 10^{o}C **(Fig. 5).** Where as at 8% NaCl, counts of the pathogen decreased. Differant isolated strains exhibited varying growth rates and tolerance ranges over the two periods of incubation. Optimum NaCl levels seems to be 0-2 NaCl for the different isolated strains of *E. coli* 0157:H7 on the basis of the maximum counts that was observed at these levels. Other investigations indicated that *E.coli* 0157:H7 was moderately salt tolerant and survives at 6.5% NaCl **(Glass *et al* 1992 & Gibson and Roberts, 1986)**. Also, **Guraya *et al* (1998)** observed *E.coli* population reduction in 6% salted

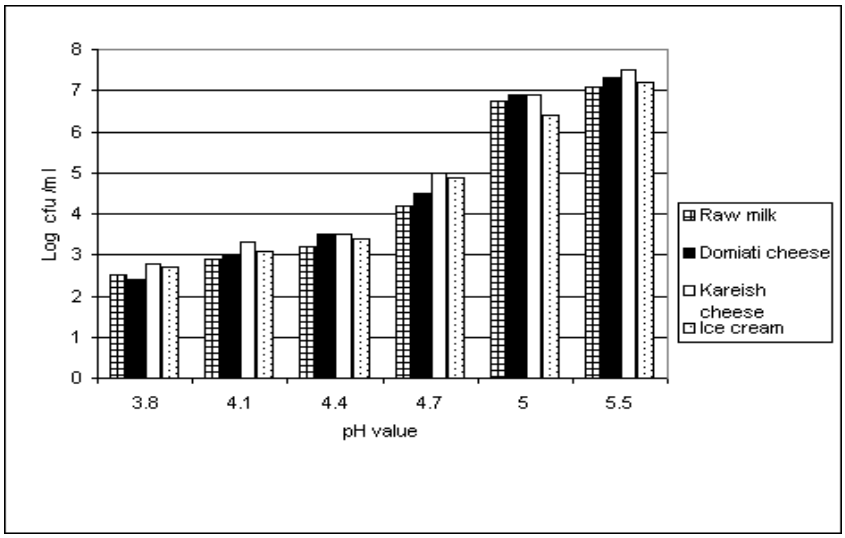

Fig. 3. Growth response of isolated strains of non-0157:H7 *E. coli* in TBS at varying pH levels at 10°C for 2 days.

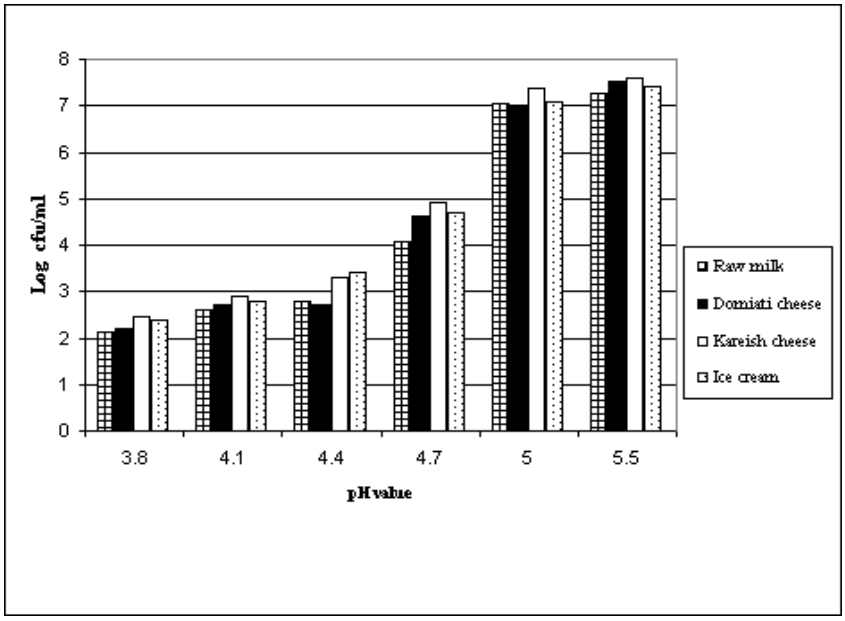

Fig. 4. Growth response of isolated strains of non-0157:H7 *E. coli* in TBS at varying pH levels at 10°C for 2 days.

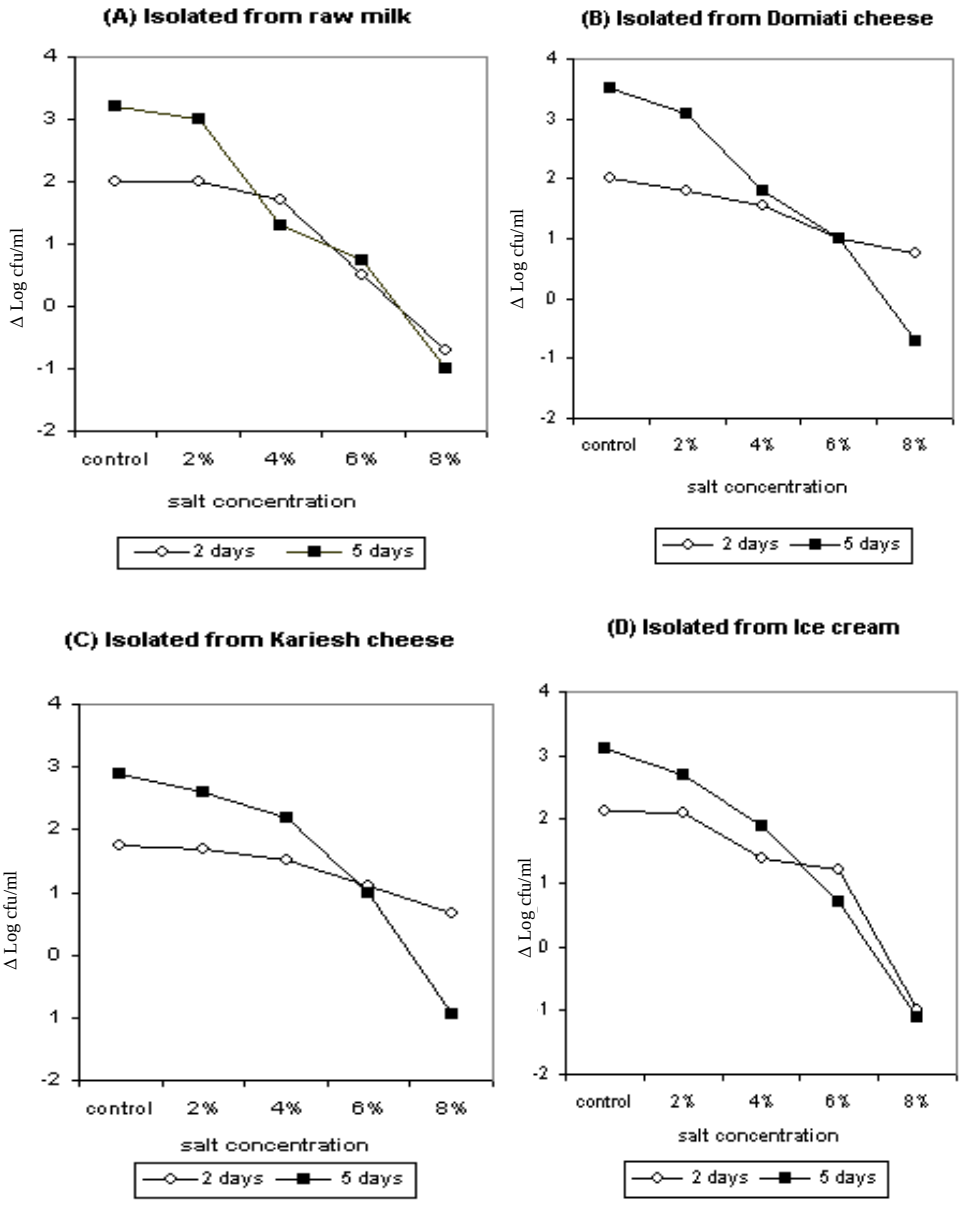

Fig. 5. Behavior of isolated strains of *E. coli* 0157:H7 in TSB at varying concentration of NaCl for 2 and 5 days at 10°C.

dairy food samples at pH 5 and 5.4. They reported that there was greater survival at lower salt levels of 2 and 4%.

The isolated strains of non-157 *E. coli* behaved similarly with different concentrations of NaCl to TSB during the incubation periods of 2 and 5 days at 10°C strains grew in NaCl different concentrations up to 6%, but a reduction of viable counts was appeared at 8%NaCl, as shown in **Fig. (6).** These results are in agreement with those obtained by **Conner and Hall (1996)** who reported that 6 or 8%NaCl in TSB showed a pronounced effect on growth of the pathognic *E. coli* during storage at 10°C.

Low temperature effects

The ability of the isolated strains of *E. coli* 0157:H7 and non-157 *E. coli* to grow at low temperature in TSB as maximum populations counts at the lowest temperature are shown in **Table (2).** All of the tested strains grew well at 10°C and increased in numbers in average more than 3 log cycles of the four strains of *E. coli* 0157:H7 and non-157 *E. coli* in a holding period of 10-days. At 8°C, all of the four strains of *E. coli* 0157:H7 were able to grow and attain maximum counts in 5-7 days. While isolates of non-157 *E. coli* attained maximum counts after 7-10 days of holding period; except the *E. coli* strains isolated from ice cream which reached maximum populations after 5 days. This is may be attributed to the adaptation of these strains to grow of low temperatures. In this concern, it has been reported that certain sub-optimum environmental condition may result in protection from subsequent stresses **(Semanchek and Golden, 1998**). However, all of the tested isolates survived during the holding period of 10-days **(Table 2).** Thus a problem might come up since some strains of enterohemorrhagic *E.coli* could grow at low temperature (8°C) and produce verotoxin **(Palumbo *et al* 1997).** There is no conflict with the finding of **Guraya *et al* (1998)** who noticed better survival at 4 °C than at 12 °C in acidic media. Also**, Massa *et al* (1999)** and **Jones *et al* (2006)** who obtained good survival of *E. coli* 0157:H7 in raw milk stored at 6 and 8°C.

Hereupon, the milk products contaminated with *E. coli* 0157:H7 and non-157 *E. coli* would remain hazard for consumption even if held at 5°C.

Table 2. Growth respones and lowest temperature for growth and survival of *E. coli* 0157:H7 and non- 0157 *E. coli* isolates.

Isolates	Lowest temperature (°C) at which growth occurred		Time (days) to attain maximum population	
	E. coli 0157:H7	Non -0157 *E.coli*	*E. coli* 0157:H7	Non -0157 *E.coli*
Milk	8	8	5	7
Domiati cheese	8	8	7	10
Kareish cheese	8	8	7	7
Ice cream	8	8	5	5

* No growth but survived at 5°C

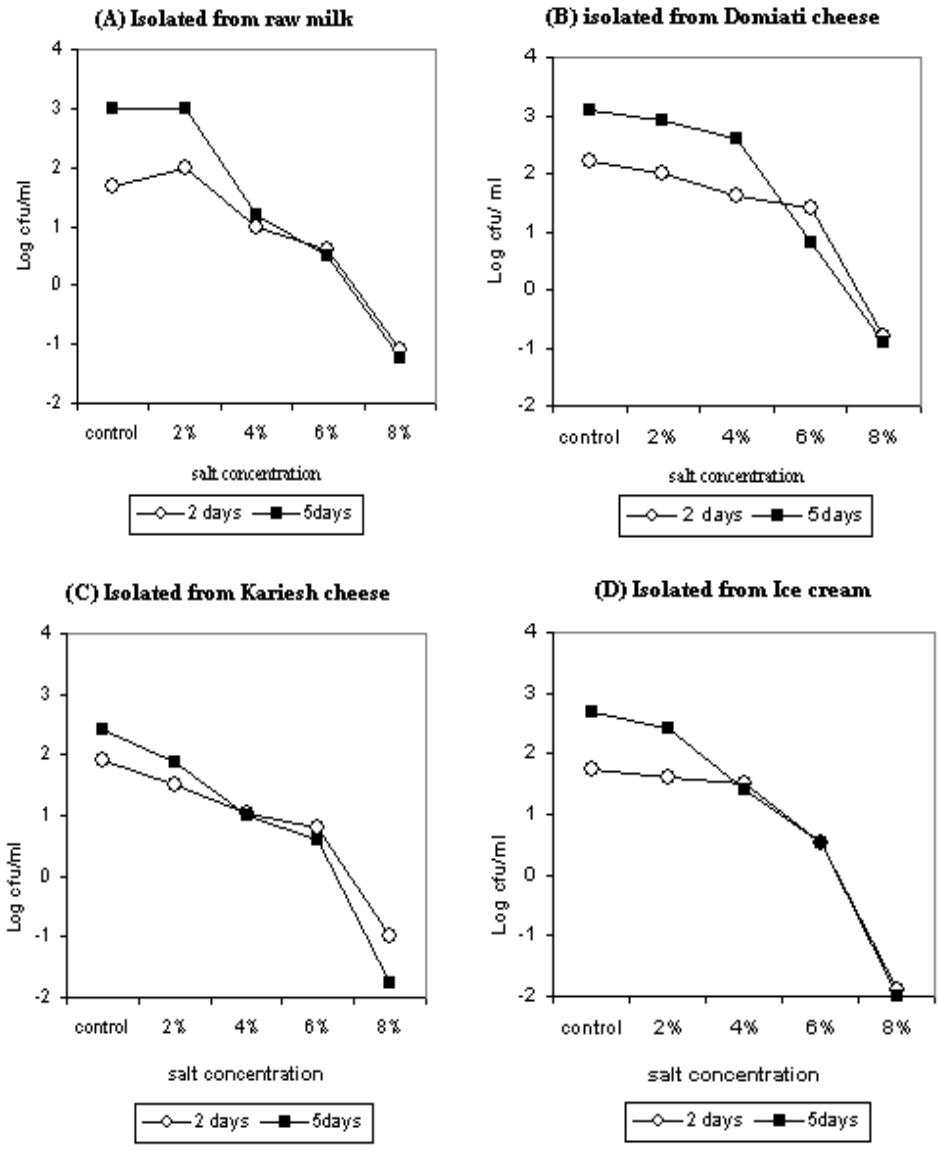

Fig. 6. Behavior of isolated strains of non- 0157:H7 *E. coli* in TSB at varying concentration of NaCl for 2 and 5 days at 10°C.

Thermal inactivation studies

Determination of heat resistance revealed that all of the tested isolates showed no heat resistance to the pasteurization temperature (65^{o}C/30 min and 72^{o}C for 15 sec.) which were sufficient to kill either of the *E. coli* 0157:H7 and non-0157 *E. coli*. These results were in agreement with those obtained by **Massa *et al* (1999)** who reported that high temperature short time (71.7^{o}C, 15 sec), was sufficient to kill approximately 1 x10^{5} *E. coli* 0157:H7 ml^{-1}.

Also the different strains of *E. coli* 0157:H7 and also non-157 *E. coli* vary in their tolerance to heat inactivation **(Fig. 7 & 8).**

The results obtained for D and Z values of the two selected strains belonging to *E. coli* 0157:H7 and non-157 *E. coli* are shown in **Table (3).** The recorded D-value at 55^{o}C of *E. coli* 0157:H7 ranged from 16.8 min, in saline solution to 26.5min in milk chocolate. While, D-value at 60^{o} C ranged from 3.7 to 5.2 min. for saline solution and chocolate milk, respectively. Also, D_{65} values ranged from 1.0 min. in saline solution to 1.5 min. in reconstituted dry milk and chocolate milk **(Table 3).** Thus, the resultant D values were high order in chocolate milk >milk> TSB medium> saline. This may be due to the presence of NaCl 9% in saline solution, the nutrient factors in TSB medium and the carbohydrates, protein, fat contents in milk and milk products **(Semanchek and Golden, 1998; Ahmed *et a*l 1995 and Duffy *et al* 2006).**

D-values obtained were close, but not identical to those reported by **Line *et al* (1991); Ahmed *et al* (1995); Kotrola and Conner (1997) and Huang & Juneja (2003).** This is due to the ecological difference between the strains or isolates, the methodology and different recovery media or different products concerning the heat resistance of selected strain of *E. coli*, non-0157 serotypes, result D-values were shown in **Table (3).**

The D_{55} values ranged from 19.5 to 24 min.; in saline solution and chocolate milk, respectively.

At 60^{o}C, the D values were 5.9, 6.2, 7.2 and 7.5 min. for saline solution, TSB, reconstituted milk and chocolate milk, respectively. While, increasing the heat treatment to 65 C resulted in reduction in all of the D-values when the D_{65} values ranged from 1.2 min in saline solution to 1.6 min .in chocolate milk comparing.

The obtained results D-values for the different selected strains of *E. coli* 0157:H7 and non-0157 *E. coli* are shown in **(Table 3).**

It was observed that the different strains varied in their sensitivity to lethal effecty of different heating temperatures, the same heating temp/time and heating media.

This might reflect difference in the heat resistance of strains of 0157 and non 0157 serogroups under the same condition**. Clavero and Beuchat (1996)** found that non-0157:H7 *E. coli* strains were less heat resistant than *E. coli* 0157:H7 strains. **Williams and Ingham (1997**) obtained D- value at 54°C for *E. coli* FRIK-124 was fivefold lower than the D-value of *E. coli* 0157:H7.

The Z-values were estimated by the linear regression between Log (D) and temp. were ranged from 7.8 to 8^{o}C for *E.coli* 0157:H7,and were ranged from 8.1 to 8.7^{o}C for non- 0157serotypes in different media. These values were similar to the value obtained by **Huang and**

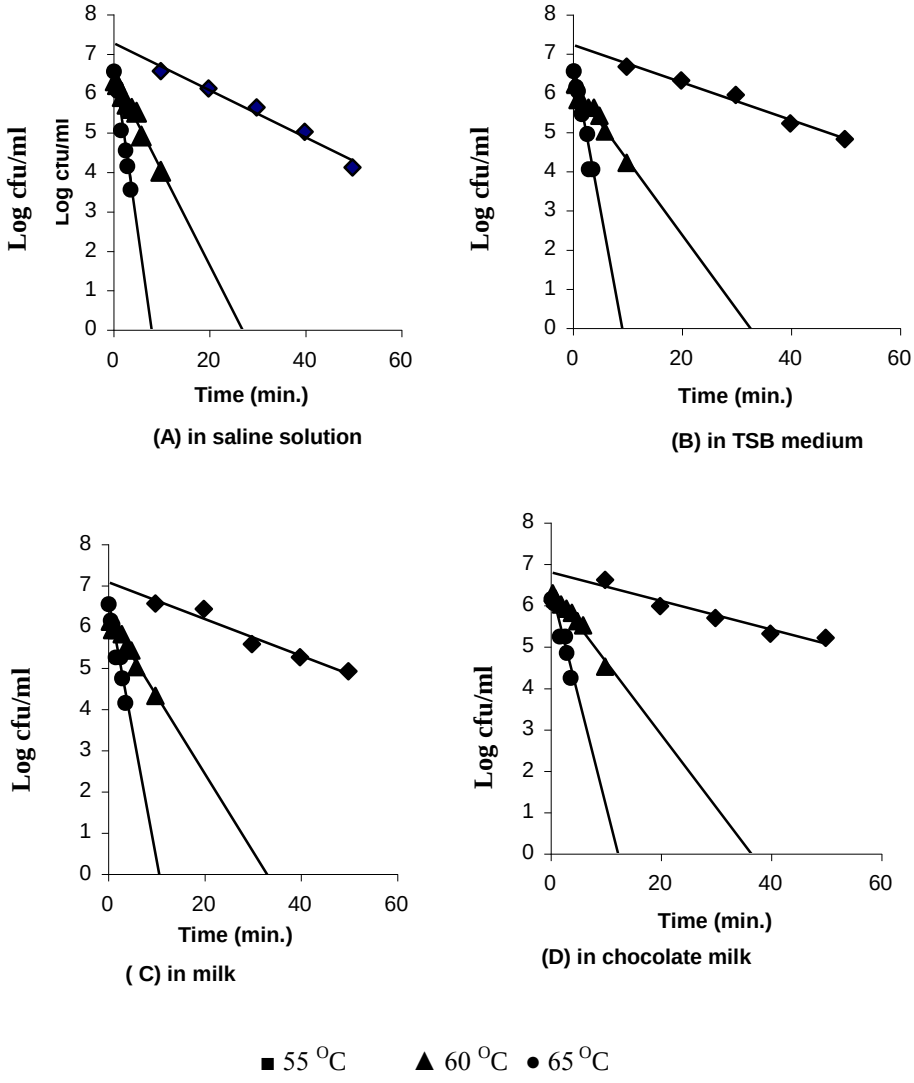

Fig. 7. Representative survivor curves of the chosen isolated E.coli 0157:H7 at different temperatures

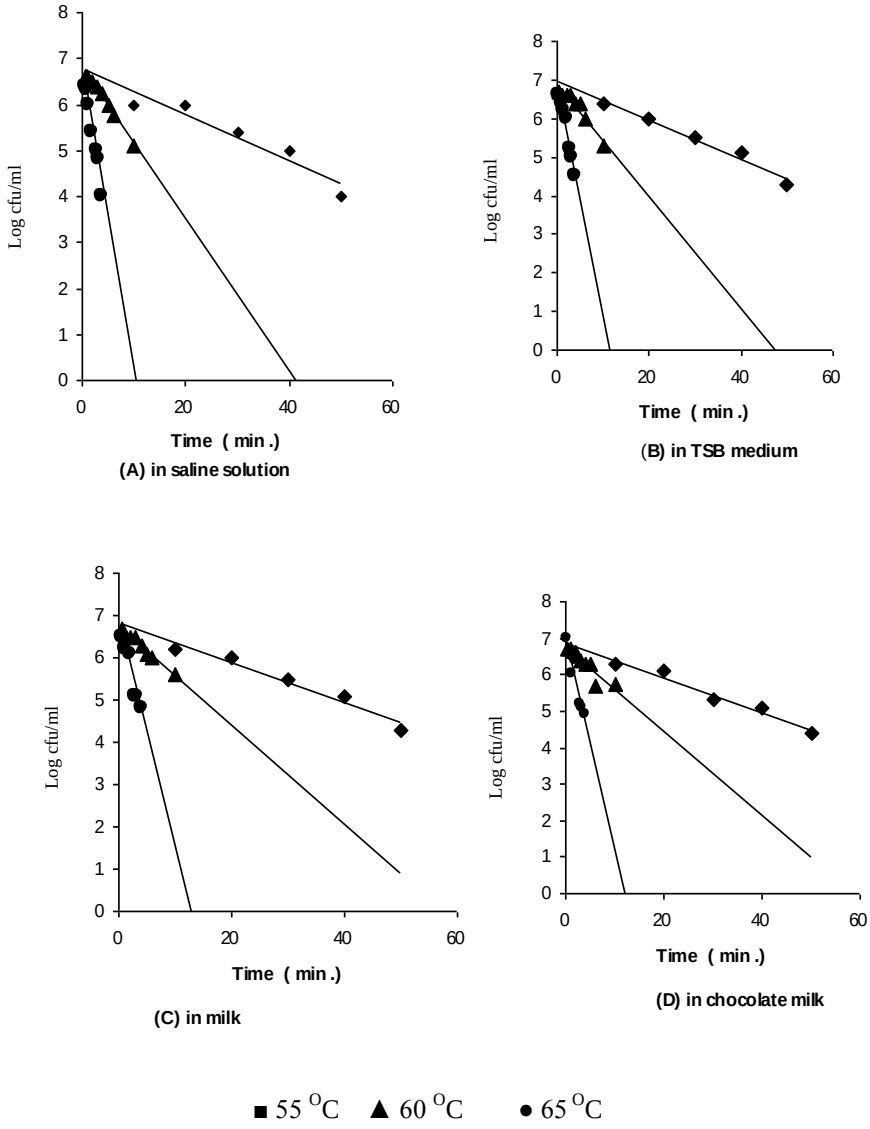

Fig. 8. Representative survivor curves of the chosen isolated non-*E.coli* 0157:H7 at different temperatures.

Table 3. D- values (minutes) and Z-values (°C) of *E.coli* 0157:H7 and non – 0157 Serogroups in different media.

Temp.	D-values (minutes)							
	E.coli 0157				non –0157 serogroups			
	Saline	TSB	Milk	Chocolate milk	Saline	TSB	Milk	Chocolate milk
55°C	16. 8	19.2	22.4	26.4	19.5	20	22	24
60°C	3.7	4.0	4.3	5.2	5.9	6.2	7.2	7.5
65°C	1.0	1.1	1.1	1.5	1.2	1.5	1.6	1.6
	Z-values (°C)							
	7.8	7.9	7.9	8.0	8.1	8.6	8.7	8.7

Juneja (2003), but higher than values (6°C) obtained by **Juneja *et al* (1997)** and (4.34-4.78°C) obtained by **Ahmed *et al* (1995).**

In conclusion, it is worthy to announce the survival and growth patterns of *E. coli* 0157:H7 and non-0157 serotypes to reduce its hazardous in milk and milk products. Milk pasteurization is queit enough to destroy these microbes and secure the customers's heath.

REFERENCES

Abd-El-Hady, H.M.; M.A. Halawa and S.H. EL-Shenawy (1995). Surveillance of enterohemorrhagic *E. coli* in milks and Kareish cheese. ***Assiut Vet. Med. J. 33: 110-112.***

Abdul-Raouf, U.M.; M.S. Ammar and L.R. Beuchat (1996). Isolation of *Escherichia coli* 0157: H7 from some Egyptian foods. ***Int. J. Food Microbiol*, *29:423-426.***

Ahmed, A.A.H.; S.H. Ahmed and M.K. Moustafa (1988). Occurrence of fecal coliforms and *enterophathogenic Escherichia* in Egyptian salt cheese***. J. Food Prot. 51: 442-444.***

Ahmed, N.H.; D.E. Conner and D.L. Huffman (1995) .Heat-resistance of *Escherichia coli* 0157:H7 in Meat and poultry as affected by product composition. ***J. Food Sci., 60: 606-610.***

American Public Health Association (A.P.H.A.) (1992). ***Compendium of Methods for the Microbiological Examination of Food. Third Ed. pp.843-845,*** American Public Health Association, Washington D.C., USA.

Arocha, M.M.; M. Mcvey; S.D. Londer; J.H. Rupnow and L. Bullerman (1992). Behavior of hemorrhagic *Escherichia coli* 0157:H7 during the manufacture of Cottage cheese***. J. Food Prot., 55: 379-381.***

Besser, R.E.; S.M. Lett; J.T. Weber; M.P. Doyle; T.J. Barrett and J.G. Wells (1993). An outbreak of Diarrhea and hemolytic uremic syndrome from fresh-pressed apple cider. ***JAMA 296: 2217.***

Clavero, M.R. and L.R. Beuchat (1996). Survival of *Escherichia coli* 0157:H7 in broth and processed Salami as influenced by pH,water activity,and temperature and suitability of media for its recovery. ***Appl. Environ. Microbial. 62: 2735-2740.***

Conner, D.E. and G.S. Hall (1996). Temperature and food additives affect growth and survival of *Escherichia coli* 0157:H7 in poultry meat. ***Dairy,Food and Environmental Sanitation 16: 150-153.***

Donnelly, C.W. and E.H. Briggs (1987). Comparison of heat resistance of *Listeria monocytogenes* in milk by two methods. ***J. Food Prot. 50: 14-19.***

Duffy, G.; C. Walsh; I.S. Blair and D.A.M.C. Dowell (2006). Survival of antibiotic resistant an antibiotic sensitive strains of *E. coli* 0157 and *E. coli* 026 in food matrices. ***Int. J. Food Microbiol 109: 179-186.***

Eblen, D.R.; B.A. Annous and G.M. Sapers (2005). Studies to select appropriate nonpathogenic surrogate *Escherichia coli* strains for potential use in place of *Esherichia coli* 0157:H7 and salmonella in pilot plant studies***. J. Food Prot., 68: 282-291.***

El-Ashmawy, M.A.M.; M. El-Sherbini and A. Abd El-Khalak (2005). The prevalence of verocytotoxigenic *Escherichia coli* and its significance in milk and some diary products. 4th ***Int. Sci. Conf. Mansoura ,1187-1197.***

El-Nokrashy, S.; A.G. Hegazi; N.F. Tawfeek; L. Aly; M.A. El-Shenawy; B.A. Effat and R.K. El-Dairouty (1992). comparative study on selective media used in recovering enteropathogenic *E. coli.* ***J. Egypt Vet. Med. Ass. 52: 483-492.***

Gibson, A.M. and T.A. Roberts (1986). The effect of pH, Water activity,sodium nitrate and storge temperature on the growth of Enteropathogenic *Esherichia coli* and salmonella in a laboratory medium***. Int. J. Food Micobiol., 6:155-178.***

Glass, K.A.; J.M. Loefielholz; J.P. Ford and M.P. Doyle (1992). Fate of *Escherichia coli* 0157:H7 as affected by pH or sodium chloride and in fermented dry sousage. ***.Appl. Environ. Microbiol. 58: 2513-2516.***

Glatz, B.A. and S.A. Brudvig (1980). Survey of commercially available cheese for Enterotoxigenic *Escherichia coli* ***.J. Food Prot., 43: 395-398.***

Guraya, R.; J.F. Frank and A.N. Hassan (1998). Effectiveness of Salt, pH and Diacetyle as inhibitors for *Escherichia coli* in dairy foods stored at refrigeration temperature. ***J. Food Prot.,61:1098-1102.***

Huang, L. and V.K. Juneja (2003). Thermal Inactivation of *Escherichia coli* 0157:H7 in Ground Beef supplemented with sodium lactale. ***J. Food Prot. 60: 664-667.***

Hussein, S.H. and T. Sakuma (2005). Shiga toxin – producing *Echerichia coli*: pre and postharvest control Measures to Ensure safety of Dairy cattle products***. J. Food Prot., 68: 199-207.***

Jones, T.H.; A. Murray; M. Johns; C.O. Gill and L.M. McMullen (2006). Differential expression of proteins in cold-adapted log-phase cultures of *Escherichia coli* incubated at 8, 6 or 2°C. ***Int. J. Food Microbiol., 107: 12-19.***

Juneja, V.K.; O.P. Snyder and B.S. Marmer (1997). Theremal destruction of *Escherichia coli* 0157:H7 in beef and chicken: determination of D-and Z-values. ***Int. J. Food Microbiol. 35: 231-237.***

Kotrola J.S. and D.E. Conner (1997). Heat Inactiviation of *Escherichia coli* 0157:H7 in Turkey Meat as Affected by Sodium chloride, Sodium lactate, polyphosphate, and Fat content. ***J. Food Prot. 60: 898-902*.**

Krieg, N.R. and J.G. Holt (1991). *Bergeys Manual of Systematic Bacteriology. Volume II, pp. 420–423.* Williams and Wilkins, Baltimore, USA.

Large, T.M.; S.T. Walk and T.S. Whittan (2005). Variation in Acid Resistance among shiga toxin-producing clones of pathogenic *Esherichia coli* .***Appl. and Environ. Microbiol .71:2493-2500.***

Line, J.E.; A.R Fain; A.B. Moran; L.M. Martin; R.V. Lechowich; J.M. Carosella and W.L. Brown (1991). Lethality of heat to *Escherichia coli* 0157:H7: D-value and z-value determination in ground beef. ***J. Food Prot. 54: 762-766.***

Massa, S.; C. Attieri; V. Quaranta and R. De Pace (1997). Survival of *E. coli* 0157:H7 in yoghurt during preparation and storage at 4°C. ***Lett. Appl. Microbiol., 24: 347-350***

Massa, S.; E. Goffredo; C. Attier and K. Natola (1999). Fate of *Escherichia coli* 0157:H7 in unpasteurized milk stored at 8^{o}C. ***Lett. Appl. Microbiol., 28: 89-92.***

Mckee, R.; R.H. Madden and A. Gilmour (2003). Occurrence of verocytotoxin producing *Esherichia coli* in Dairy and Meat processing environments***. J. Food Prot., 66: 1576-1580*.**

Miller, L.G. and C.W. Kaspar (1994). *Escherichia coli* 0157:H7 acid tolerance and survival in apple cider. ***J. Food Prot. 57: 460-464.***

Neill, M.A. (1997) .Overview of verotoxigenic *Escherichia coli***. *J. Food Prot., 60:1444-1446.***

Ogwaro, B.A.; H. Gibson; M. Whitehead and D.J. Hill (2002). Survival of *Escherichia coli* 0157;H7 in traditional African yoghurt fermentation***. Int. J. Food Microbiol., 79:105-112***.

Padhye, N.V. and M.P. Doyle (1992). *Escherichia coli* 0157:H7 epidemiology ,pathogenesis and methods for detection in Foods. ***J. Food Prot., 55: 555-565.***

Palumbo, S.A.; A. Pickard and J.E. Call (1997). Population changes and verotoxin production of Enterohemorrhagic *E.coli* strains inoculated in milk and ground beef held at low temperature. ***J. Food Prot., 60: 746-750.***

Reitsma, C.J. and D.R. Henning (1996). Survival of Enterohemorrhagic *Escherichia coli* 0157:H7 during manufacture and curing of Cheddar ***J. Food Prot., 59: 460-464.***

Semanchek, J.J. and D.A. Golden (1998). Influence of growth temperature on inactivation and injury of *Escherichia coli* 0157:H7 by heat, acid and freezing***. J. Food Prot., 61: 395-401*.**

Williams, N.C. and S.C. Ingham (1997). Changes in heat resistance of *Escherichia coli* 0157:H7 following heat shock***. J. Food Prot., 60: 1128-1131*.**

Zhao, T.M.; M.P. Doyle and R. Besser (1993). Fate of Enterohaemorrhagic *Escherichia coli* 0157:H7 in apple cider with and without preservatives***. Appl. Environ. Microbiol., 59: 252-2530*.**

Zadik, P.M.; P.A. Chapmanand; C.A. Siddons (1993). Use of tellurite for the selection of verocytotoxigenic *Esherichhia coli* 0157. ***J. Medical Microbiol. 39: 155-158.***

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قدرة سلالات الميكروبين *Escherichia coli* 0157:H7 و non-0157 المعزوله من منتجات الآلبان على البقاء تحت الظروف غير المناسبة للنمو [42]

زينب ابراهيم صادق[1]

1. قسم الالبان المركز القومى للبحوث- الدقى- القاهرة- مصر

تم فحص 150 عينة من اللبن الخام - الزبد- الجبن القريش-الجبن الدمياطي- الايس كريم ميكروبيولوجيا لوجود مجموعة القولون و *E. coli* وكذلك ميكروب *E. coli* 0157:H7 و non-0157 المنتجة للسموم والمسببة للأ سهال المدمم ،حيث تم عزل سلالات الميكروبين الاخيرين من عينات اللبن الخام و الجبن القريش و الجبن الدمياطى بنسب تتراوح بين 6.6% الى 20% من اجمالى العينات كما وجد ان اعلى نسبة تلوث بهذين الميكروبين كانت في عينات الجبن القريش.

بدراسة سلوكها وقدرتها علي النمو والبقاء في وجود فى وجود تركيزات مختلفة من الحموضة والملح وكذلك درجات الحرارة المنخفضة وتأثير المعامله الحرارية، وجد ان كلا الميكروبين يستطيع البقاء على pH 3,8 مع امكانية النمو في وجود تركيزات من الملح تصل الى 6% هذا بجانب قدرتها على البقاء على درجة حرارة ٥°م. و بتقدير معاييرالمقاومة الحراريه D-and Z- values للسلالات التى تم اختيارها حسب مقاومتها الحرارية و ذلك عند تلقيحها في محلول ملحى وبيئة ببتون صويا السائله (TSB) ولبن جاف مسترجع ولبن شوكولاته، أظهرت النتائج أن أعلى قيمة لكلا من Z- and D- values قد سجلت في لبن الشيكولاته بالنسبة للسلالتين وكما لوحظ أن نوع السلاله وطبيعة المنتج له تأثيرعلى المقاومة الحرارية للميكروب ولذا فان البستره هى السبيل الامثل للقضاء على هذة الميكروبات وضمان تأمين صحة المستهلك.

تحكيم: ا.د محمد نبيل إبراهيم المجدوب
ا.د نبيل فؤاد توفيق