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FATE OF SHIGATOXIGENIC AND NON-TOXIGENIC STRAINS OF *ESCHERICHIA COLI* IN RAW, PASTEURIZED AND BOILED MILK STORED AT DIFFERENT TEMPERATURES

By

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ABSTRACT

Shiga toxin producing *Escherichia coli* (STEC) is the most important group of newly emerged food-borne pathogens. Therefore, the present study was conducted to trace the survivability of shigtoxigenic, non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains inoculated separately into raw, pasteurized and boiled milk with initial inoculums of 10^5 cfu/ml. and kept at different storage temperatures (4, 8 and 12 °C) and time. The results indicate good survival or even multiplication of *E. coli* O157 in milk when stored at 8 and 12 °C that reaffirms the need for pasteurization and holding the milk at 4 °C.

Key words:

Shigatoxin producing *E. coli*, fate, *E. coli* O157, milk.

INTRODUCTION

Shiga toxin producing *E. coli* (STEC) or enterohaemoragic *E. coli* (EHEC) were first identified as human pathogens in 1982, after occurrence of hemorrhagic colitis outbreaks due to consumption of undercooked hamburgers contaminated with *E. coli* O157:H7. The symptoms associated with STEC infection in humans vary from mild to bloody diarrhea (hemorrhagic colitis), which is often accompanied by abdominal cramps, usually without fever. STEC infections can result in hemolytic uremic syndrome (HUS) which is characterized by acute renal malfunction and failure, microangiopathic hemolytic anemia (lysis of red blood cells), and thrombocytopenia (lowered platelet counts). Hemolytic Uremic Syndrome developed in up to 10% of patients infected with STEC O157 that lead to acute renal failure in young children; it can also result in seizures, coma and sometimes death **(McCrae and Cines, 2000, Bacon and Sofos, 2003 and Beauchamp and Sofos, 2010).** Thrombotic thrombocytopenic purpura (TTP) is a form of HUS typically developed by the

elderly and includes fever, platelet loss and neurological symptoms **(Lawley *et al.,* 2008)** Non-O157 infections may induce a range of other illnesses, from mild gastroenteritis to critical illness, and death, either as sporadic cases or in outbreaks. Due to inadequate analytical methods and epidemiological and laboratory surveillance, non-O157 STEC infections may be under-recognized and under-reported around the world **(Hanna *et al.,* 2010).** STEC are usually associated with foods derived from cattle such as milk and dairy products as the bovine intestinal tract is a known reservoir of *E. coli* O157:H7. Transmission of the pathogen from cattle to humans occurs via faecal contamination of food and water, as well as through direct contact, person-to-person spread via the faecal-oral route has also occurred causing outbreaks in institutions and child-care settings such as nurseries. STEC might also enter the milk supply from mastitic cows where the mastitis was caused by *E. coli* **(Lira *et al.,* 2004).**VTEC can grow over the temperature range 7-46 °C with an optimum of 37 °C. Some isolates of *E. coli* O157:H7 grow poorly at 44-45 °C, so that traditional methods to detect *E. coli* in food may not pick up this important pathogen. VTEC survive well at chilled and frozen temperatures. Low temperature is reported to be the primary trigger for VTEC to enter a ‘‘viable non-culturable’’ state (VNC) in water. STEC O157:H7 have been shown to survive at temperatures that are lower than those are suitable for growth. For example, *E. coli* O157:H7 can survive for several weeks or months in ice cream and yogurt when frozen at -18 to -20 °C **(Grzadkowska and Griffiths, 2001; De Schrijver *et al.,* 2008; Strawn and Danyluk, 2010 and Farrokh *et al.,* 2012).** The organism is destroyed by pasteurization and killed at 64.3 °C in 9.6 s but the cells survive well in food at - 20 °C **(Bhunia, 2008).** The prevention and control of *E. coli* O157:H7 infection requires strict hygienic measures at all stages of the food chain. Therefore, awareness should be created among consumers about the importance of hygienic practices in the preparation, handling and storage of food items in the control and prevention of this newly emerging pathogen. In addition, an implementation of effective management procedures should be practiced **(Engdaw and Temesgen, 2016).**

MATERIAL AND METHODS

Used milk:

Fresh whole buffalo's milk (proved raw by Guaiac test**, Schoenberg, 1956)** was obtained

from a small dairy farm, and proved free from any inhibitory substances as by Lactic Acid Activity Test **(Kosikowski and Mistry, 1997)**.

Used strains:

Three strains of previously identified *E. coli* were used:

1- *E. coli* O157:H7 reference strain ATTCC No. (51659) obtained from Cairo MIRCEN, Faculty of Agriculture, and Ain Shams University.

2- Shigatoxigenic *E. coli* non O157: Isolated and identified by PCR.

3- Non shigatoxigenic *E. coli* O157: Isolated and identified by PCR.

The inoculum of *E. coli* strains were cultured overnight in brain heart infusion broth (BHI) at 37 °C according to **(Mamani *et al.,* 2003)**. Serial dilutions of BHI cultures of the microorganisms were made in sterile peptone water. The population of each serial dilution was determined spectrophotometrically and by surface plating onto Plate Count Agar [PCA].

Raw milk:

Raw milk was subjected to Guaiac test **(Schonberg, 1956)** for elimination of milk proved heat-treated. Before inoculation, total bacterial count of raw milk was determined according to **ISO, 2002**.

Pasteurized milk:

Laboratory pasteurization of milk was adopted according to **Egdell *et al.,* (1950),** as the milk heated to 63°C for 30 min. in thermostatically controlled water bath, followed by detection of efficiency of pasteurization by Alkaline phosphatase test using Lactognost test kit (Heyl Chemisch-pharmazeutische Fabrik GmbH and Co. KG, Berlin, Germany).

Boiled milk:

Boiling of milk was performed according to **(Metwally *et al.,* 2011),** as the milk was heated at 100 °C for 10 min**.,** efficiency of boiling was detected by Guaiac test **(Schonberg, 1956).**

Inoculation of milk with microorganisms:

Bacterial culture (previously prepared shigatoxigenic *E. coli* O157, shigatoxigenic *E. coli* non O157 and non shigatoxigenic *E. coli* O157 strains) inoculated individually into 500 ml. milk (raw, pasteurized and boiled milk) at final conc. of 10^5 cfu/ml., and dispensed into sterile tubes **(Wang *et al.,* 1997)**. All tubes (inoculated milk and control milk) were incubated at 4 °C, 8 °C and 12 °C. The experiment was carried out in triplicates according to **Palumpo *et al.,* 1997; Wang *et al.,* 1997 and Massa *et al.,* 1999.** Samples were examined on days

0, 1, 3 and every 2 days to determine pH, acidity percentage, pathogen survival and detection of shiga toxin production.

Chemical examination:

Measurement of pH value was applied according to method NO. 981.12 Of **AOAC (2003)**, using a digital pH meter with a probe type combined electrode (HANNA, HI 98/30 waterproof pH meter).Three readings were recorded and the average was calculated. Titratable acidity percentage of milk was adopted according to **APHA, 2004**.

Microbiological examination:

Preparation of food homogenate, decimal dilutions and enumeration of *E. coli* using spreading technique were adopted as mentioned by **APHA, 2004.**Total bacterial count of milk was adopted according to **ISO, 2002.**

Table (1): Recovery of *E. coli* strains during storage of raw milk at 4 °C.

Time	Control			Shigatoxigenic *E. coli* O157				Non-toxigenic *E. coli* O157				Shigatoxigenic non O157 *E. coli*			
	A %	pH	TCC Log cfu/ml.	A%	pH	TCC Log cfu/ml.	Count Log cfu/ml.	A%	pH	TCC Log cfu/ml.	Count Log cfu/ml.	A%	pH	TCC Log cfu/ml.	Count Log cfu/ml.
Zero time (inoculated milk)	0.14	6.65	8.43	0.14	6.65	14.86	5.59	0.14	6.65	13.08	5.99	0.14	6.65	14.04	5.34
24 hour	0.15	6.62	8.04	0.17	6.57	14.11	5.59	0.17	6.55	13.34	5.04	0.18	6.53	14.92	5.57
3 days	0.17	6.58	8.85	0.18	6.41	13.73	5.54	0.18	6.47	13.81	5.53	0.20	6.46	14.08	5.60
5 days	0.19	6.45	8.41	0.22	6.36	13.43	5.38	0.21	6.38	12.28	5.08	0.29	6.32	13.83	5.51
7 days	0.27	6.23	7.48	0.31	6.19	12.34	5.18	0.30	6.17	11.89	4.78	0.36	6.20	12.11	5.23

- A=Acidity.
- TCC= Total colony count.

Table (2): Recovery of *E. coli* strains during storage of raw milk at 8 °C.

Time	Control			Shigatoxigenic *E. coli* O157				Non-toxigenic *E. coli* O157				Shigatoxigenic non O157 *E. coli*			
	A %	pH	TCC Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml
Zero time (inoculated milk)	0.14	6.65	8.43	0.14	6.65	14.86	5.59	0.14	6.6 5	14.07	5.99	0.14	6.6 5	14.04	5.34
24 hours	0.28	5.95	8.79	0.27	5.95	15.52	6.75	0.24	6.11	15.60	6.04	0.26	6.01	14.72	6.95

Table (3): Recovery of *E. coli* strains during storage of raw milk at 12 °C.

Time	Control			Shigatoxigenic *E. coli* O157				Non-toxigenic *E. coli* O157				Shigatoxigenic non O157 *E. coli*			
	A %	pH	TCC Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml
Zero time (inoculated milk)	0.14	6.65	8.43	0.14	6.65	14.86	5.59	0.14	6.65	14.08	5.99	0.14	6.65	14.04	5.34
24 hours	0.38	5.63	9.56	0.41	5.55	16.15	6.98	0.40	5.60	16.98	6.83	0.39	5.58	16.99	6.83

Table (4): Recovery of *E. coli* strains during storage of Pasteurized milk at 4 °C.

Time	Control			Shigatoxigenic *E. coli* O157				Non-toxigenic *E. coli* O157				Shigatoxigenic non O157 *E. coli*			
	A %	pH	TCC Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml
Zero time (inoculated milk)	0.14	6.58	2.28	0.15	6.56	8.41	5.99	0.15	6.56	8.04	5.84	0.14	6.58	8.58	5.78
24 hour	0.14	6.57	2.40	0.16	6.54	8.40	5.99	0.15	6.54	8.36	5.81	0.15	6.55	8.60	5.76
3 days	0.15	6.55	2.86	0.16	6.53	8.56	5.91	0.17	6.50	8.48	5.72	0.16	6.53	8.73	5.57
5 days	0.16	6.51	2.93	0.17	6.50	8.88	5.89	0.19	6.45	8.78	5.11	0.18	6.45	8.90	5.46
7 days	0.16	6.51	2.61	0.18	6.48	8.81	5.15	0.20	6.41	7.49	5.04	0.18	6.42	8.92	5.26
9 days	0.17	6.46	2.20	0.20	6.40	8.79	5.04	0.22	6.38	7.00	4.93	0.20	6.38	8.51	5.04
12 days	0.17	6.44	1.67	0.22	6.36	8.94	4.86	0.25	6.35	6.88	4.41	0.23	6.30	8.20	4.08
15 days	0.20	6.40	1.36	0.26	6.29	7.51	4.72	0.27	6.30	6.71	4.00	0.28	6.23	7.85	3.87
18 days	0.23	6.32	1.36	0.29	6.19	7.18	4.54	0.30	5.98	6.70	3.56	0.30	6.11	7.62	3.62

Table (5): Recovery of *E. coli* strains during storage of Pasteurized milk at 8 °C.

Time	Control			Shigatoxigenic *E. coli* O157				Non-toxigenic *E. coli* O157				Shigatoxigenic non O157 *E. coli*			
	A %	pH	TCC Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml
Zero time (inoculated milk)	0.14	6.58	2.28	0.15	6.56	8.41	5.99	0.15	6.56	8.04	5.84	0.14	6.58	8.58	5.78
24 hour	0.16	6.55	3.40	0.17	6.53	9.66	6.58	0.17	6.51	8.49	6.20	0.16	6.53	8.71	6.26
3 days	0.17	6.52	3.60	0.18	6.50	9.88	6.83	0.19	6.48	9.70	6.77	0.17	6.43	9.51	6.59
5 days	0.18	6.50	3.97	0.18	6.49	9.97	6.93	0.20	6.46	9.91	6.79	0.18	6.41	9.48	6.95
7 days	0.19	6.48	3.48	0.19	6.45	9.83	6.89	0.23	6.40	10.72	7.56	0.20	6.37	9.56	7.27
9 days	0.19	6.46	2.90	0.20	6.38	8.49	5.56	0.27	6.35	10.04	7.83	0.22	6.32	9.00	6.08
12 days	0.21	6.33	2.26	0.29	6.23	7.96	5.04	0.30	6.26	8.87	6.52	0.39	6.11	8.32	5.81
15 days	0.24	6.27	1.88	0.38	5.90	7.60	5.70	0.35	6.09	7.79	4.97	0.42	5.95	7.89	4.46

Table (6): Recovery of *E. coli* strains during storage of Pasteurized milk at 12 °C.

Time	Control			Shigatoxigenic *E. coli* O157				Non-toxigenic *E. coli* O157				Shigatoxigenic non O157 *E. coli*			
	A %	pH	TCC Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml
Zero time (inoculated milk)	0.14	6.58	2.28	0.15	6.56	8.41	5.99	0.15	6.56	8.04	5.84	0.14	6.58	8.58	5.78
24 hour	0.17	6.53	3.81	0.18	6.46	10.91	6.96	0.17	6.50	9.91	6.72	0.18	6.47	10.80	6.95
3 days	0.19	6.32	3.84	0.23	6.15	10.97	7.15	0.21	5.96	10.67	7.88	0.35	5.17	10.93	7.11

Table (7): Recovery of *E. coli* strains during storage of boiled milk at 4 °C.

Time	Control			Shigatoxigenic *E. coli* O157				Non-toxigenic *E. coli* O157				Shigatoxigenic non O157 *E. coli*			
	A %	pH	TCC Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml
Zero time (inoculated milk)	0.15	6.65	1.08	0.15	6.65	6.38	5.18	0.15	6.67	6.30	5.70	0.14	6.54	6.26	5.48
24 hour	0.16	6.52	1.23	0.16	6.50	6.45	5.23	0.16	6.56	6.45	5.70	0.16	6.50	6.40	5.51
3 days	0.18	6.48	1.40	0.19	6.43	6.63	5.15	0.18	6.44	6.76	5.61	0.17	6.42	6.79	5.41
6 days	0.20	6.42	1.65	0.22	6.38	6.92	5.00	0.19	6.41	6.94	5.61	0.19	6.30	6.96	5.40
9 days	0.23	6.39	1.49	0.26	6.30	5.52	4.96	0.22	6.33	6.63	4.43	0.22	6.27	5.79	4.73
12 days	0.25	6.33	1.18	0.30	6.11	2.32	------	0.27	5.89	6.04	-----	0.26	6.05	2.08	-----

Table (8): Recovery of *E. coli* strains during storage of boiled milk at 8 °C.

Time	Control			Shigatoxigenic *E. coli* O157				Non-toxigenic *E. coli* O157				Shigatoxigenic non O157 *E. coli*			
	A %	pH	TCC Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml
Zero time (inoculated milk)	0.15	6.65	1.08	0.15	6.65	6.38	5.18	0.15	6.67	6.30	5.45	0.14	6.54	6.26	5.48
24 hours	0.16	6.58	1.38	0.17	6.56	6.63	5.45	0.17	6.58	6.67	5.60	0.17	6.49	6.49	5.58
3 days	0.18	6.42	1.88	0.19	6.40	7.11	5.78	0.18	6.46	7.48	5.87	0.19	6.37	7.38	5.72
6 days	0.20	6.38	2.04	0.22	6.36	7.93	6.18	0.20	6.32	7.88	6.08	0.21	6.30	7.83	6.60
9 days	0.23	6.29	2.32	0.25	6.15	8.80	6.82	0.37	5.68	8.60	6.18	0.30	5.92	8.91	6.62
12 days	0.25	6.18	2.41	0.29	6.07	7.91	5.70	0.39	5.61	6.85	4.41	0.32	5.87	6.60	4.00
15 days	0.29	5.97	2.38	0.33	5.85	2.48	-------	0.40	5.58	2.32	-----	0.34	5.82	2.20	------

j.Egypt.net.med.Assoc 76, no 2. 199 – 214 (2016)

Table (9): Recovery of *E. coli* strains during storage of boiled milk at 12 °C.

Time	Control			Shigatoxigenic *E. coli* O157				Non-toxigenic *E. coli* O157				Shigatoxigenic non O157 *E. coli*			
	A %	pH	TCC Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml	A%	pH	TCC Log cfu/ml	Count Log cfu/ml
Zero time (inoculated milk)	0.15	6.65	1.08	0.15	6.65	6.38	5.18	0.15	6.67	6.30	5.70	0.14	6.54	6.26	5.48
24 hours	0.18	6.57	1.45	0.18	6.53	6.68	6.15	0.18	6.49	6.53	6.15	0.18	6.46	6.70	6.18
3 days	0.20	6.36	2.20	0.20	6.29	7.60	6.40	0.20	6.33	7.48	6.48	0.24	6.31	7.63	6.53
6 days	0.20	6.27	2.34	0.21	6.21	7.97	6.53	0.30	6.07	7.90	6.04	0.28	6.19	7.90	6.11
9 days	0.22	6.23	2.67	0.24	6.09	7.62	6.81	0.32	5.97	7.86	6.70	0.30	5.82	7.79	6.41
12 days	0.24	6.17	2.26	0.29	5.93	7.34	5.90	0.36	5.65	6.15	4.92	0.33	5.73	6.48	5.34
15 days	0.28	6.05	2.04	0.35	5.81	2.30	-------	0.39	5.58	4.30	3.30	0.40	5.68	4.61	3.90

DISCUSSION

E. coli O157:H7 has no unusual heat resistance; however, a number of factors, including pH, growth conditions and growth phase of the cells, and the method of heating **(Riemann and Cliver, 2006)**, can influence the thermal resistance. Data depicted in (Table 1) showed the behavior of shigatoxigenic, non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains in raw milk stored at 4°C with initial counts of 5.59, 5.99 and 5.34 log cfu/ml., respectively. The initial acidity percentage and pH value of the milk were 0.14% and 6.65, respectively. The initial total colony count (TCC) of 8.43 log cfu/ml. Shigatoxigenic, non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains had a little change in the count reaching 5.18, 4.78 and 5.23 log cfu/ml., respectively, at titratable acidity of 0.31, 0.30 % and 0.36, respectively, and pH values of 6.19, 6.17 and 6.20, respectively. By day 7, TCC decreased to 7.48 log cfu/ml. in control sample (after 7 days of storage, the milk became unfit for human consumption).**Wang *et al.* (1997) and Giacometti *et al.* (2012)** detected similar results**,** while longer **Tremonte *et al.* (2014)** obtained survival time. Toxigenic and non-toxigenic *E. coli* strains were unable to multiply or produce verocytotoxins if the raw milk was kept refrigerated at the correct temperature (5°C) **(Massa *et al.,* 1999).** On the other hand, *E. coli* O157:H7 survived well in refrigerated raw milk and dairy products as the organism could survive for long time at low temperature than at high temperature, **(McClure and Hall, 2000)**. (Table2) illustrated that in raw milk stored at 8°C, the count of shigatoxigenic, non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains continuously increased after 24 hours to 6.75, 6.04 and 6.95 log cfu/ml., respectively, with an increase in titratable acidity to 0.27, 0.24, and 0.26%, respectively, while pH values were 5.95, 6.11, and 6.01, respectively. While in control milk samples there were slight increase in TCC to 8.79 log cfu/ml. after storage for 24 hours at 8°C (the milk became unfit for human consumption). These findings were in agreement with what have been reported by **Massa *et al.,* 1999.** He found that good survival or even multiplication of *E. coli* O157:H7 in raw milk stored at 8° C revealed continuous increase in the counts of shigatoxigenic, non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains to 6.98, 6.83 and 6.83 log cfu/ml., respectively (Table 3). After 24 hours of raw milk storage at 12 °C, titratable acidity were 0.41, 0.40 and 0.39%, respectively, while pH values were 5.55, 5.60 and 5.58, respectively. TCC increased to 9.56 log cfu/ml. after 1day of raw milk storage at 12°C in

control milk sample (the milk became unfit for human consumption). **Palumbo *et al.* (1997) and Timm *et al.* (2009)** obtained nearly similar results, while **Massa *et al.* (1999)** obtained longer survival period. *E. coli* strains used in this trial were seen to be good competitors with the microbial population of the unpasteurized milk, which increased in all samples at 8 and 12°C. Moreover, it appears that these *E coli* O157:H7 strains were not inactivated by lactoperoxidase system, which in raw milk, in combination with H_2O_2 and SCN, produces metabolites that are bactericidal to many Gram –negative bacteria **(Farrag and Marth 1992 and Massa *et al.* 1999)**. The shift in pH also failed to inhibit growth of the organism; these paralleled with those of **Massa *et al.* (1999)**. The presence and the possible multiplication of *E. coli* strains in raw milk reaffirms the need for pasteurization, as the high temperature short time process which is commonly used to pasteurize milk, is sufficient to kill approximately $1X10^5$ *E. coli* O157:H7 ml^{-1} **(D'Aoust *et al.*, 1988)**. Data depicted in (Table 4) demonstrated the behavior of shigatoxigenic, non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains in pasteurized milk held at 4°C with initial counts of 5.99, 5.84 and 5.78 log cfu/ml., respectively. The initial titratable acidity and pH values in the milk were 0.14% and 6.58, respectively; the total colony count (TCC) of the control-pasteurized milk was 2.28 log cfu/ml. The organisms could survive for 18 days in refrigerated pasteurized milk, shigatoxigenic *E. coli* O157, non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains showed continuous decrease in the count reaching 4.54, 3.56 and 3.62 log cfu/ml, respectively, at titratable acidity of 0.29, 0.30 and 0.30%, respectively, and pH values of 6.19, 5.98 and 6.11, respectively. By the 18^{th} day, TCC decreased to 1.36 log cfu/ml. in control milk. After 18 days of storage, the milk became unfit for human consumption. In pasteurized milk stored at 8°C, shigatoxigenic, non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains could survive for 15days. The counts of Shigatoxigenic, non-toxigenic *E. coli* O157 , shigatoxigenic and O157 *E. coli* strains showed continuous increase until day 5 (6.93 log cfu/ml), day 9 (7.83 log cfu/ml) and day 7 (7.27 log cfu/ml), respectively. Then the counts continuously decreased until day 15 with counts of 5.70, 4.97 and 4.46 log cfu/ml, respectively, with increase in titratable acidity to 0.38, 0.35 and 0.42%, respectively, pH values were decreased to 5.90, 6.09 and 5.95, respectively. By the day 15, TCC decreased to 7.60 log cfu/ml in the control pasteurized milk sample (The milk became unfit for human consumption after 15 days of storage).(Table 5).The viability of Shigatoxigenic non-toxigenic

E. coli O157 and shigatoxigenic non 157 *E. coli* strains in pasteurized milk stored at 12°C, showed that the organisms could survive for 3 days with continuous increase in counts reaching 7.15, 7.88 and 7.11 log cfu/ml, respectively, with an increase in titratable acidity to 0.23, 0.21 and 0.35%, respectively, and decrease in pH values to 6.15, 5.96 and 5.17, respectively (Table 6),. TCC increased to 3.84 log cfu/ml in control milk (The milk became unfit for human consumption after 3 days of storage at 12°C). Despite the fact that in the real circumstance, the consumers buy and drink the milk within few days after placing in the store, the temperature should be below 4ºC for preventing the propagation of bacteria. A little prolongation of a standard pasteurization temperature is recommended to be applied in raw milk, without the destruction of milk properties. In addition, the storage of pasteurized milk in the refrigerated shelves under 4ºC is also recommended to prevent the propagation of contaminated pathogens **(Pewleang *et al.*, 2013).** Results from (Table 7) illustrated the behavior of shigatoxigenic, non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains in boiled milk held at 4°C with initial counts of 5.18, 5.70 and 5.48 log cfu/ml., respectively. The initial titratable acidity and pH values in the milk were 0.15% and 6.65, respectively; the total colony count (TCC) of boiled milk was 1.08-log cfu/ml. After 24 hours of storage, shigatoxigenic *E. coli* O157 and non O157 counts increased slightly to 5.23 and 5.51 log cfu/ml., respectively. On the other hand, non-toxigenic strain of *E. coli* O157 maintained under non-growth conditions with the same initial count (5.70 log cfu/ml.), then all *E. coli* strains continuously decreased until they could not be detected at 12th day of storage at titratable acidity of 0.30, 0.27 and 0.26%, respectively, and pH value of 6.11, 5.89 and 6.05, respectively. TCC of control and inoculated milk continuously increased until the 6th day of storage, and then decreased till the 12th day of storage.However, in boiled milk stored at 8°C, shigatoxigenic, non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains showed continuous increase until the 9th day of storage reaching maximum counts of 6.82, 6.18 and 6.62-log cfu/ml, respectively. After that, the counts continuously decreased until they could not be detected at the 15th day of storage, the titratable acidity values were 0.33, 0.40 and 0.34%, respectively, and pH values were 5.85, 5.58 and 5.82, respectively. TCC slightly increased until the 12th day of storage and then decreased to reach 2.38-log cfu/ml in control milk at the 15th day of storage, while in inoculated milk, TCC continuously increased until the 9th day of storage and then decreased until the end of storage

period. (Table 8). Results demonstrated in (Table 9), revealed the survival of shigatoxigenic *E. coli* O157 until the 12th day of storage in boiled milk stored at 12°C with count of 5.90 log cfu/ml. The titratable acidity and pH value were 0.29% and 5.93, respectively, while non-toxigenic *E. coli* O157 and shigatoxigenic non O157 *E. coli* strains could be detected until the 15th day of storage with counts of 3.30 and 3.90 log cfu/ml., respectively, and titratable acidity of 0.39 and 0.40%, respectively, and pH values of 5.58 and 5.68, respectively. TCC showed continuous increase in control and inoculated milk at the 9th day of storage, and then decreased until the end of storage period (After 15 days of storage, milk became unfit for consumption). **Mamani *et al.* (2003) and Pewleang *et al.* (2013)** obtained nearly similar results. The total coliform decreased during storage period due to the inhibitory effect of increased acid production **(Tamime and Robinson, 1999)**. Shiga toxin producing *E. coli* could survive well in raw milk and dairy products as well as low pH products. The usual associated illness includes diarrhea, which often bloody and abdominal cramps, with little or no fever, illness usually lasted for 6-8 days and is more pronounced in the young and elderly. A serious complication of infection with *E. coli* O157:H7 is the Hemolytic Uremic Syndrome (HUS) which characterized by renal failure, microangiopathic hemolytic anemia where the red blood cells are smaller than normal and have shortened survival and severe thrombocytopenic purpura (reduction in the number of blood platelets) and the death rate is ranged from 3-8%**(WHO, 1999).** Contamination of milk and dairy products with these microorganisms because of bad sanitary measures during their production, handling and distribution associated with the absence or inadequate monitoring; therefore, the results of the present study warn the need for more meticulous preventive measures to improve the quality of milk and its products and to safeguard consumers from being infected.

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