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EFFECT OF HEAT TREATMENT ON THE SURVIVAL OF *Escherichia coli* O157:H7 IN RAW MILK

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ABSTRACT

The survival of E*scherichia coli* O157:H7 in raw milk treated in experimental pasteurizer was investigated in the year 2010. Raw milk was inoculated with different initial concentrations of *E. coli* O157:H7 and heated for 15 seconds at temperatures ranging from 69^{O}C to 73^{O}C. *E. coli* O157:H7 cells were not isolated from the milk samples immediately after thermal treatment. They were however, isolated from 72% of heated samples after variable periods of storage at refrigeration temperature (4^{O}C). The result suggests that a low number of *E. coli* O157:H7 survived some thermal treatment, but a cold storage in the refrigerator would have been responsible for repairing the thermally injured cells and thus detect this pathogen in milk. The results also revealed that the lower the initial counts of *E. coli* O157:H7 in raw milk samples the lower their percentage recovery from the heat treated milk. As the infectious dose of *E. coli* O157:H7 for human is very low, the presence of the organism in milk even in low numbers, is a potential risk for the consumers because this would lead to clinical infection associated with it.

KEYWORDS: *E. coli* O157:H7, Thermal treatment, Cold storage, Pathogen, Infectious dose.

INTRODUCTION

There are different strains of *Escherichia coli* present in nature. They are usually found in the intestines of healthy humans and animals. Even though these bacteria offer beneficial properties, there are some strains that are pathogenic (Feng *et al*., 2000). *Escherichia coli* O157:H7 is one particular strain that is an emerging cause of food-borne illness (Benjamin and Datta, 1995). This pathogen first identified as a human pathogen in 1982 in Oregon and Michigan, United States of America, causes severe disease including hemorrhagic colitis (bloody diarrhoea), hemolytic uremic syndrome (HUS) (that leads to acute kidney failure) and thrombotic thrombocytopenic purpura (that can be fatal, and involves loss of platelets, skin colourations, fever and nervous disorder in addition to HUS signs and symptoms) (Doyle, 1991, Griffin and Tauxe, 1991).

Although *E. coli* O157:H7 has been isolated from a wide range of sources such as salad vegetables, weaning cereal pastes and beef (Abdul Raouf, 1993; Mawak and Ashamu 2006; Dahiru *et al*., 2008), foods of bovine origin have been identified or suspected as the principal vehicle in most outbreaks (Doyle, 1991; Griffin and Tauxe, 1991). According to Hayes *et al*. (1986), Morgan *et al*. (1993) and Anon (2003) raw milk and yoghurt have also been identified as sources of food-borne illness caused by *E. coli* O157:H7. Apart from the fact that *E. coli* O157:H7 causes diseases, its presence in milk in large numbers can cause rapid spoilage of raw milk and milk products (UK National Dairy Council, 1981)

Buchanan and Doyle (1997) reported that *E. coli* O157:H7 can be controlled readily through traditional thermal processing techniques. However, the organism's low infectious dose (Makino *et al*., 2000) requires that processing be sufficient to assure a low probability of the pathogen surviving. According to Buchanan and Doyle (1997) dairy pasteurization processes designed to kill spore forming bacteria such as *Bacillus* spp. and *Clostridium* spp. should be sufficient to eliminate *E. coli* O157:H7. Contrary to this report, this pathogen and other pathogenic bacteria like *Listeria monocytogenes* have also been implicated in outbreaks due to the consumption of commercially pasteurized milk (Fernandez *et al*., 1986; McDonough *et al*., 1991). According to U.K, National Dairy Council (1981) effective pasteurization ensures the destruction of pathogenic organisms, but if they are detected in heat treated milk, they originate from either inadequate pasteurization or post pasteurization contamination.

As pasteurization techniques can affect the survival of *E. coli* O157:H7, the aim of the present work was to study the thermal resistance of this pathogen in an experimental pasteurizer.

MATERIALS AND METHODS

Preparation of Inoculum

Escherichia coli serotype O157:H7 isolated randomly from twenty one samples of raw milk in Microbiology laboratory of the division of Plant Science and Technology, University of Jos, was used for this experiment. Jos is located in the central part of Nigeria between latitudes 8^{O}.30$^{'}$ with 10^{O} 10$^{'}$N and longitudes 8^{O}.20' and 9^{O}.30'E with a surface area of about 9,400km^{2}. To prepare an inoculum the culture was grown in 10mls of Tryptone Soya Broth (TSB) (Oxoid, CMO 129) for 2 hours at 37^{O}C, centrifuged, and the sediment suspended in 10mls phosphate buffer. This suspension was then used as the primary inoculum (Naylor and Sharpe, 1998). In order to allow the adaptation of *E. coli* O157:H7 to the milk, 10ml of the

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inoculum was added to 90ml of sterile raw milk (heated in water bath at 85^{O}C for 30min (Fernandez *et al*., 1987). Further double fold serial dilutions were made to obtain cell concentration of approximately 10^8 cfu/ml using TSB.

Preparation of Milk Samples

Four litres of raw milk samples were obtained from a local farm and transported to the laboratory, where they were stored in the refrigerator at 4^{O}C for about one hour prior to use. The milk samples were sterilized by heating in water bath at 85^{O}C for 30min and cooled with chilled water at 1^{O}C (Fernandez *et al*., 1987). A measured volume (90ml) of each of the sterile milk sample
s (divided into 5 batches; M1, M2, M3, M4 and M5) was dispensed into sterile stainless steel containers covered with aluminum foil, after which 10ml each of the inoculum was added into the 5 batches of the milk samples. This process was repeated until 30 portions were obtained for each batch. After this, further serial dilutions of the contaminated milk samples (M1, M2, M3, M4 and M5) were made to obtain cell concentrations of 8.0 7.0, 6.0, 5.0, and 4.0 $\log_{10}$cfu/ml respectively. These cell concentrations were the initial *E. coli* O157:H7 counts in the various milk samples.

Thermal Treatments and Isolation of *E. coli* O157:H7

The thermal resistance of *E. coli* was assayed using a water bath. The milk samples (M1, M2, M3, M4 and M5) were then subjected to high temperature treatments of 69, 70, 71, 72 and 73^{O}C for 15 seconds. A thermometer was used to monitor the temperature by making a hole in the center of the foil and inserting the thermometer through it into the milk menstrum (Hayes, 1986).

After thermal treatment and subsequent cooling at 1^{O}C, all milk samples were stored in the refrigerator at 4^{O}C for 5days. Counts were made on the refrigerated samples for the period of 5 days, after duplicate surface plating on cefixime tellurite-sorbitol MacConkey (CT-SMAC) agar medium and incubation at 37^{O}C for 18-24 hours. From each positive milk sample, five colonies were subcultured and confirmed by biochemical analysis for the presence of *E. coli* O157:H7 using the criteria proposed by Cheesbrough (1991).

Contaminated milk samples of each of the batches, which did not receive heat treatment were used as controls.

Statistical Analysis

Each of the heat treatment was repeated five times and the means of the bacterial counts that were obtained from the milk samples during cold storage in the refrigerator were subjected to analysis of variance (ANOVA). Multiple comparison of the treatment was done using Duncan's Range Test.

RESULTS

The results of the assessment of the thermal resistant of *Escherichia coli* O157 H7 in milk samples are shown in figures 1 – 5. *E. coli* O157:H7 was isolated from 72% of the heated milk samples, although never immediately after thermal treatment, but only after variable periods of cold storage at refrigeration temperature. However, all samples heated at 73^{O}C and samples with initial counts of 4.0 and 5.0 $\log_{10}$cfulml (M5 and M4 respectively) heated at 72 ^{O}C for 15 seconds did not yield *E. coli* O157:H7 during the 5 days storage at refrigeration temperature.

Results in Figures 1 – 5 revealed that all the milk samples heated at 69^{O}C and 70^{O}C for 15 Seconds became contaminated a day after storage in the refrigerator with contamination levels ranging from 2.9 x 10^2 to 3.8 x 10^2 cfu/ml in M1 samples, 1.2 x 10^2 to 2.7 x 10^2cfu/ml in M2 samples, 0.9 x 10^2 to 1.8 x 10^2 cfu/ml in M3 samples, 0.5 x 10^2 to 0.9 x 10^2 cfu/ml in M4 samples and 0.3 x 10^2 to 0.5 x 10^2 in M5 samples. Out of all the milk samples heated at 71^{O}C, 60% became contaminated on the second day with contamination levels ranging from 0.5 x 10^2 to 1.5 x 10^2 cfu/ml (Figures 1 -3), while 40% became contaminated on the third day with contamination levels ranging from 0.2 x 10^2 to 0.6 x 10^2 (Figures 4 and 5). *E. coli* O157:H7 was only isolated from M1, M2 and M3 samples, heated at 72^{O}C after refrigeration for 72hrs with contamination levels reaching 6.4 x 10^2cfu/ml, 5.5 x 10^2 cfu/ml and 3.2x 10^2 cfu/ml on day 5 respectively (Figures 1 – 3). Thus, 25% of the heated milk which were positive for *E. coli* O157:H7 were from milk heated at 72^{O}C for 15sec, a thermal treatment prescribed for high temperature short time (HTST) pasteurization process.

The results from this study showed that the higher the heating temperatures, the lower the level of bacterial contamination of the milk samples during cold storage in the refrigerator. It was also observed from this study that on day 5 period of cold storage in refrigerator, a significantly ($P<0.05$) higher viable population of *E. coli* O157: H7 cells were recovered from most of the milk samples positive with the pathogen than those recovered on the first 3 days .

The results of the comparison between the effects of different initial counts of *E. coli* O157: H7 on its percentage recovery from milk samples stored under refrigeration temperature after heat treatment process are shown in Table1. The results showed that the higher the initial counts of *E. coli* O157:H7, the greater its percentage recovery from heat treated milk stored at refrigeration temperature for 5 days.

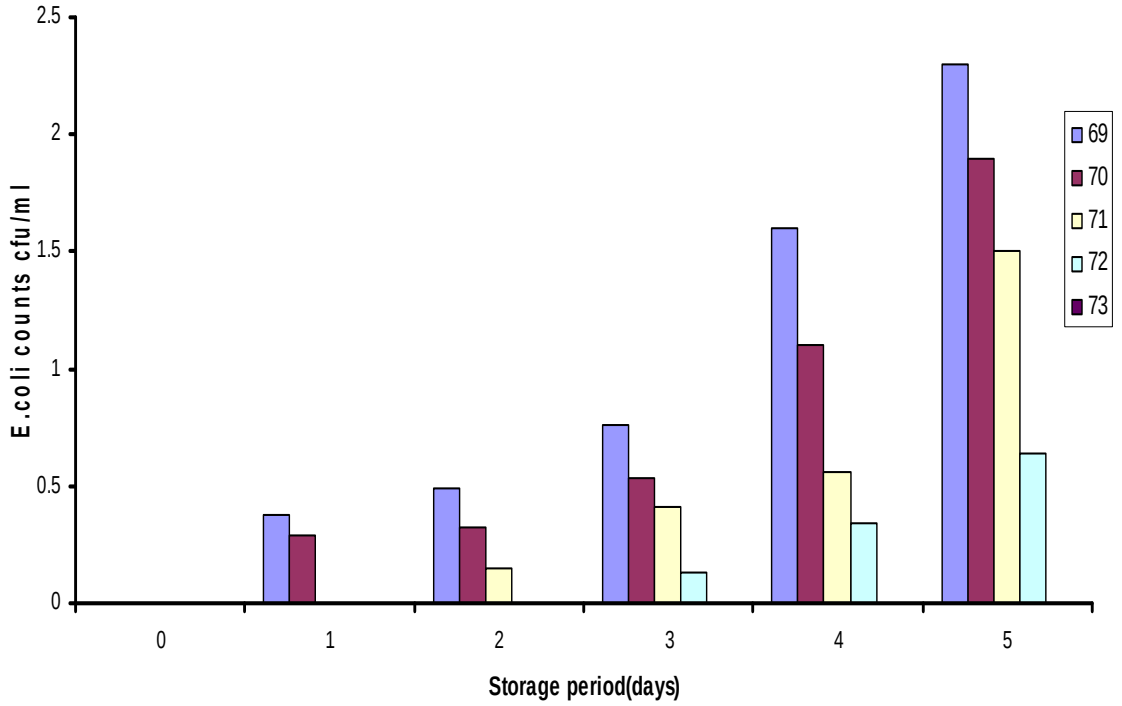

Fig 1: *E. coli* O157:H7 counts from milk samples (M1 with initial count of 8.0 $\log_{10}$ cfu/ml) stored at refrigeration temperature (4^0C) during the first 5 days after heat treatment (69-73^{O}C/15 sec)

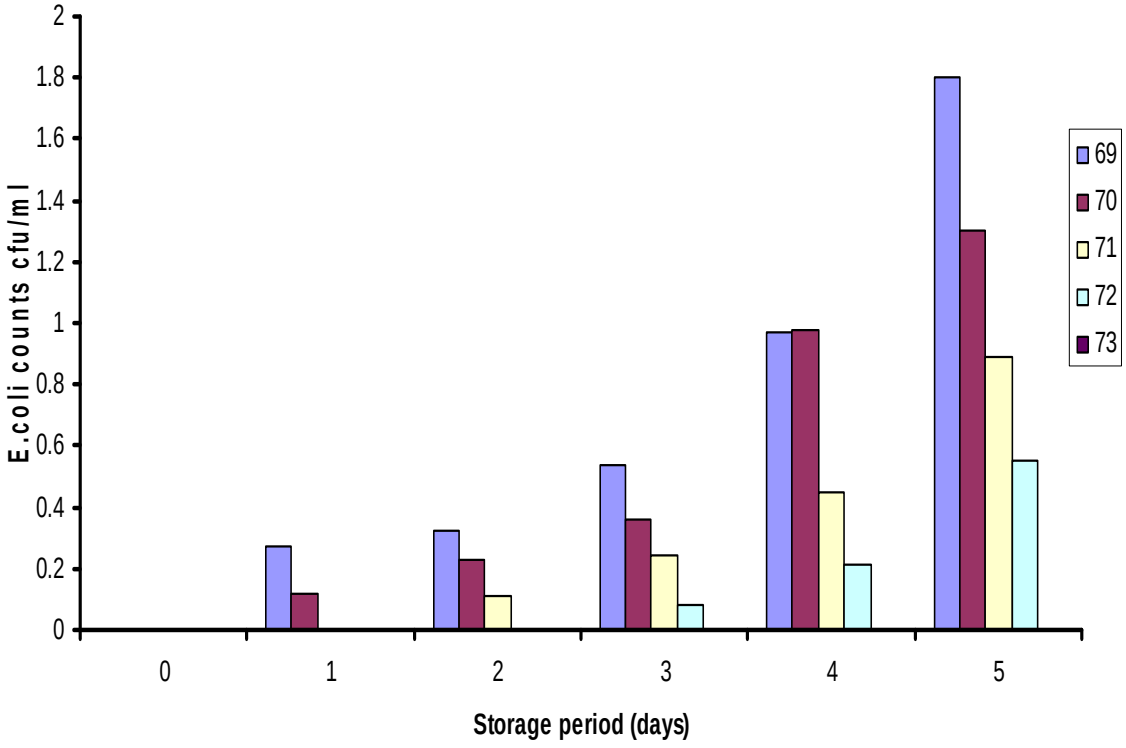

Fig 2: *E. coli* O157:H7 counts from milk samples (M2 with initial count of 7.0 $\log_{10}$ cfu/ml) stored at refrigeration temperature (4^0C) during the first 5 days after heat treatment (69-73^{O}C/15 sec)

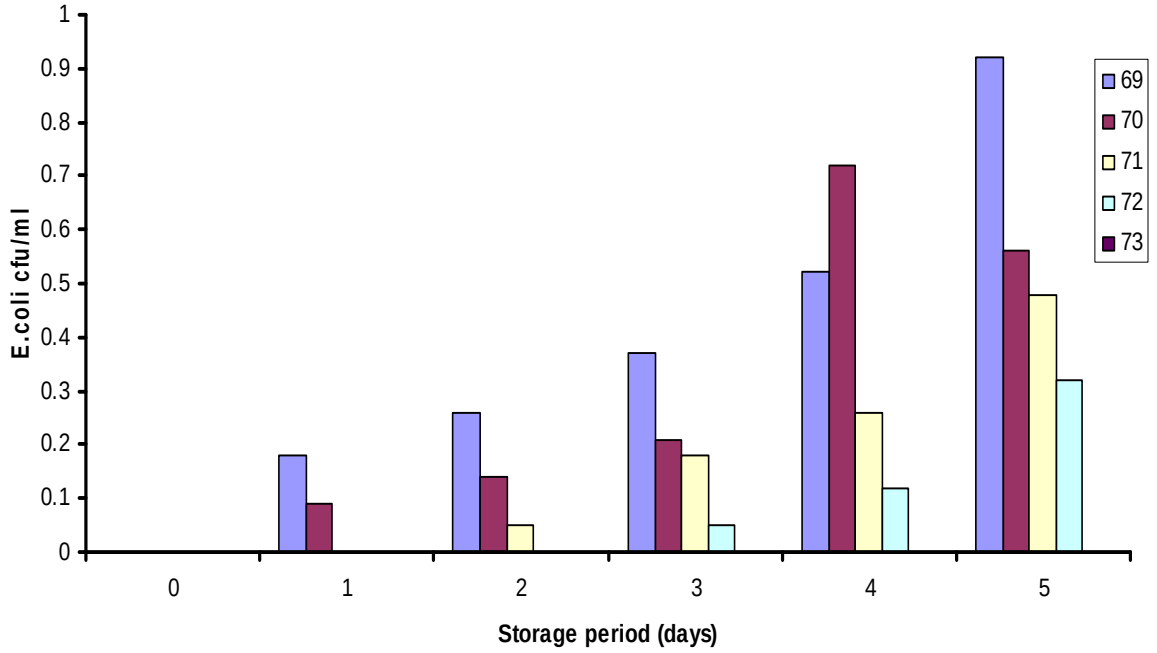

Fig 3: *E. coli* O157:H7 counts from milk samples (M3 with initial count of 6.0 $\log_{10}$ cfu/ml) stored at refrigeration temperature (4^0C) during the first 5 days after heat treatment (69-73^{O}C/15 sec)

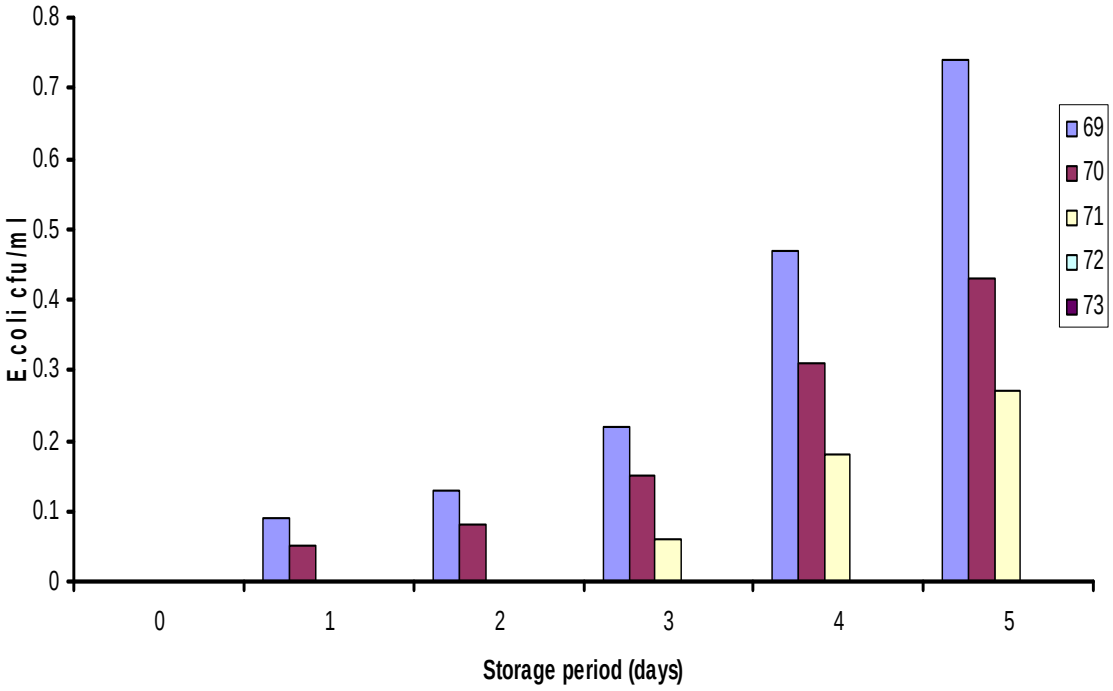

Fig 4: *E. coli* O157:H7 count from milk samples (M4 with initial count of 5.0 $\log_{10}$ cfu/ml) stored at refrigeration temperature (4^0C) during the first 5 days after heat treatment (69-73^{O}C/15 sec)

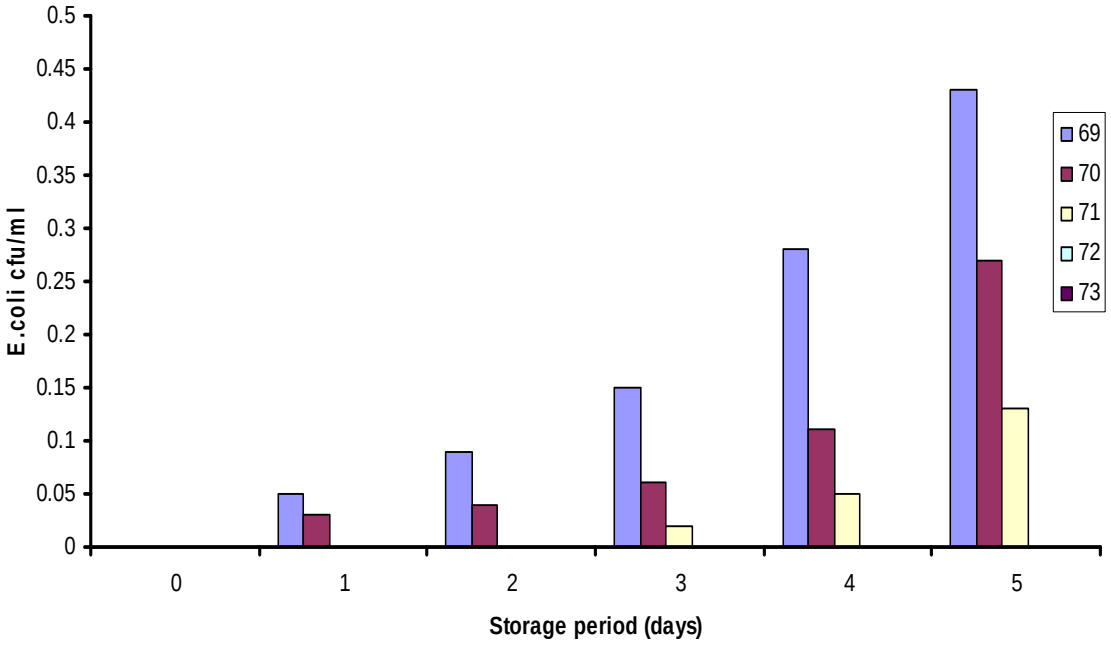

Fig 5: *E. coli* O157:H7 count from milk samples (M5 with initial count of 4.0 $\log_{10}$ cfu/ml) stored at refrigeration temperature (4^0C) during the first 5 days after heat treatment (69-73^0C/15 sec)

Table 1: Comparison between effects of different initial counts of *E. coli* O157:H7 (M1-M5) on the percentage recovery of *E. coli* O157:H7 from milk samples stored at refrigeration temperature (4^OC) for 5 days after heat treatment (69-73^OC/ 15sec)

Heating Temp.^{O}C	Storage period (days) 0	1	2	3	4	5
69						
M1	0.00a	1.27b	1.63b	2.53c	5.33d	7.67e
M2	0.00a	0.90a	1.07b	1.80b	3.23c	6.00d
M3	0.00a	0.60a	0.87a	1.23b	1.73b	3.07c
M4	0.00a	0.03a	0.43a	0.73a	1.57b	2.47c
M5	0.00a	0.02a	0.30a	0.50a	0.93a	1.43b
70						
M1	0.00a	0.97a	1.07b	1.77b	3.67c	6.33d
M2	0.00a	0.40a	0.77a	1.20b	3.27c	4.33c
M3	0.00a	0.30a	0.47a	0.70a	2.27b	1.87b
M4	0.00a	0.17a	0.27a	0.50a	0.03a	1.43b
M5	0.00a	0.10a	0.13a	0.20a	0.37a	0.90a
71						
M1	0.00a	0.00a	0.50a	1.37b	1.87b	0.50a
M2	0.00a	0.00a	0.37a	0.80a	1.50b	2.97c
M3	0.00a	0.00a	0.17a	0.60a	0.87a	1.60b
M4	0.00a	0.00a	0.00a	0.13a	0.60a	0.90a
M5	0.00a	0.00a	0.00a	0.07a	0.17a	0.43a
72						
M1	0.00a	0.00a	0.00a	0.43a	1.13b	2.13c
M2	0.00a	0.00a	0.00a	0.27a	0.37b	1.83c
M3	0.00a	0.00a	0.00a	0.17a	0.46b	1.07c
M4	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
M5	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
73						
M1	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
M2	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
M3	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
M4	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
M5	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a

Means in the same column with similar letters are not significantly different at 5% level of probability using DMRT

DISCUSSION

The numbers of *E. coli* O157:H7 recovered from heat treated milk greatly depended on the initial population of the pathogen present before pasteurization, the temperature of the heat treatment and the duration of storage in cold refrigerator. The composition of milk makes it not only an excellent food for man but also an ideal medium for the growth of pathogenic bacteria and other microorganisms. However previous reports by Donnely and Briggs (1986) and Donnelly *et al*. (1987) indicated that pasteurization ensures the destruction of pathogenic organisms in milk. The fact that no *E. coli* O157:H7 was isolated from any of the milk samples immediately after thermal treatment would explain and agree with the findings of these authors. Results from this study, however, suggest that during thermal treatments most *E. coli* O157:H7 cells were inactivated, although a number of survivors remain in the heated milk due to possible intracellular localization. A cold storage may have been responsible to repair thermally-injured cells and to protect this microorganism in milk. This suggestion agrees with previous work (Dominguez *et al*., 1986b) in which bacterial species like *Listeria* spp were recovered from inoculated raw whole milk heated at 72^{O}C for 15 sec only after cold storage. Buchanan and Klawitter (1992) also reported that low temperature storage enhanced the survival of *E. coli* O157:H7 in foods after heat treatments.

The results from the present study reveal that the high temperature short time (HTST) pasteurization technique (72^{O}C/15 sec) recommended by Food and Drug Administration (1978) did not completely eliminate the *E. coli* O157:H7 from milk samples. This result is supported by McDonough *et al*. 1991) who reported that commercial pasteurized milk has been identified as a source of food-borne illness caused by *E. coli* O157:H7. Present study also has shown that differences in heating temperature can have significant effect on the destruction of *E. coli* O157:H7 cells, thus the higher the heating temperatures the more the organism decline in number. This could explain why the organism was not recovered from milk samples heated at 73^{O}C for 15 sec even during cold storage under refrigerator for up to 5 days. However, this temperature (73^{O}C) is above the recommended temperature for (HTST) (72^{O}C) pasteurization process. The implications of this is well explained by the report of UK National Dairy Council (1981), which stated that pasteurization process has very little effect on milk flavour and the nutritional value; however, over heating will affect both qualities of milk.

The results of this study showed that the higher the initial population of *E. coli* O157:H7 in milk subjected to heat treatments, the higher the level of contamination of the milk by the organism during cold storage at refrigeration temperature. Thus, the numbers that survive heat treatments could multiply in milk reaching levels potentially hazardous once consumed. This results in transmission of infections caused by this pathogen, especially when refrigeration temperatures are not low enough. Fernandez *et al*. (1987) also observed in his experiment that when the initial *Listeria monocytogenes* population in milk was high, the numbers that survived thermal treatment of pasteurization multiplied during storage at refrigeration temperatures. According to UK National Dairy Council (1981) the initial population of raw milk can be reduced when milk is drawn aseptically from the udder of a healthy cow. This reduces the number of microorganisms to a minimal level that can easily be destroyed through effective pasteurization, thus presenting no danger to the consumer. The present study also reveals that the percentage recovery of *E. coli* O157:H7 in raw milk (without heat treatments) far exceed ($p<0.05$) those subjected heat treatment process (Table1). Donnelly and Briggs (1986) also reported an increase in population of *Listeria monocytogenes* from 16cfu/ml to 1.2×10^{4} and 5.8×10^{6} cfu/ml, within 24 and 48h (respectively) in raw whole milk kept at 10^{O}C.

Isolation of *E. coli* O157:H7 from milk after heat treatment is an indication that more studies should be done to determine the exact risk of its food-borne transmission. However, in the present investigation, attention has been drawn to the possible health, sanitary and economic interest and to the importance of initial population of *E. coli* O157:H7 in milk subjected to heat treatment at various temperatures and subsequent storage in refrigeration temperature.

CONCLUSION

As the infectious dose of *E. coli* O157:H7 for human is low, its presence in pasteurized milk even in low numbers, is a potential risk for the consumer because it would lead to intestinal colonization and, in certain situations such as immunosuppressive illnesses or therapies, to the development of clinical infections caused by this pathogen. In addition, the presence of *E. coli* strains such as *E. coli* O157:H7 in milk or dairy products can also cause rapid spoilage of milk, which will eventually lead to great economic loses. It has become necessary that raw milk should not only be properly processed before consumption, good handling procedures should also be employed by the farmers during milking process to reduce the initial counts of *E. coli* O157:H7 in raw milk.

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