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PREVALENCE OF ENTEROHEMORRHAGIC ESCHERICHIA COLI O157:H7 IN RAW MILK AND EFFECT OF SOME CHEMICAL PRESERVATIVES ON ITS VIABILITY

(With One Table and 4 Figures)

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مدى تواجد ميكروب الايشريكية القولونية المسببة للالتهاب المعوي النازف في اللبن الخام تأثير بعض المواد الحافظة على حيويتها

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يعتبر اللبن غذاء صحي متكامل لكافة مراحل العمر وذلك لما يحتويه من عناصر غذائية هامة تجعله ذو قيمة عالية إلا أنه قد يتعرض للتلوث من مصادر مختلفة أثناء الإنتاج أو التداول مما يؤثر على جودته نتيجة تغيرات غير مرغوبة تجعله غير صالح للاستهلاك الآدمي. تم تجميع عدد ١٠٠ عينة من اللبن الخام من المزارع و محلات الألبان و الباعة الجائلين بمحافظتى الإسكندرية والبحيرة لعزل ميكروب الايشريكية القولونية عترة O157:H7 المسببة للالتهاب المعوي النازف. وقد اسفرت النتائج على تلوث العينات بميكروب الايشريكية القولونية عترة O157:H7 بنسبة ١%. وتم مناقشة الأهمية الصحية العامة للميكروب. كما تم دراسة تأثير بعض المواد الحافظة على حيويتها في اللبن المعقم مثل سوربات البوتاسيوم(٠,١٤٠,٠٥%) وبنزوات الصوديوم(٠,١٤٠,٠٥%) واستخدام سوربات البوتاسيوم وبنزوات الصوديوم معا بتركيز ٠,١% لكل منها في اللبن المعقم و نظام اللاكتوبيروكسيدز المنشط في اللبن المعالج بالحرارة دون البسترة. ووجد أن بنزوات الصوديوم (٠,١%) لها القدرة على القضاء على الميكروب بعد ثمانية أيام من التخزين في درجة حرارة الثلاجة ووجد أن سوربات البوتاسيوم تقلل من العدد بمقدار لوغاريتم واحد فقط. ولكن عند استخدام سوربات البوتاسيوم (٠,١%) وبنزوات الصوديوم(٠,١%) معا فانها تقضي على الميكروب بعد ستة أيام من التخزين في درجة حرارة الثلاجة. أما بالنسبة الى نظام اللاكتوبيروكسيدز المنشط في اللبن المعالج بالحرارة دون البسترة ووجد أنه يقلل من العدد بمقدار اثنان لوغاريتم.

SUMMARY

E. coli O157:H7, an Enterohemorrhagic pathogen, causes hemolytic uremic syndrome and hemorrhagic colitis in human. The objective of this study was determination of the prevalence of E. coli O157:H7 in raw milk and to evaluate the inhibitory effect of sodium benzoate, potassium sorbate, combination of sodium benzoate and potassium sorbate, and activated Lactoperoxidase system (LPS) on the viability of E. coli O157:H7 in commercially sterile milk stored at 4 °C. One hundred samples of raw milk were collected randomly from different areas in Alexandria and El-Behera Governorates, Egypt. The collected samples were examined for the prevalence of E. coli O157:H7 using Sorbitol MacConkey agar and rapid latex agglutination test for identification of E. coli serogroup O157. Different concentrations of Sodium benzoate (0.05 & 0.1%); Potassium sorbate (0.05 & 0.1%), combination of Sodium benzoate (0.1%) and Potassium sorbate (0.1%), and Lactoperoxidase system were added to detect their effects on E. coli O157:H7 viability. E. coli O157:H7 was detected in 1% of the examined samples. Sodium benzoate (0.1%) eliminates E. coli O157:H7 after 8 days and Potassium sorbate (0.1%) reduces E. coli O157:H7 one log cfu/ml after 10 days. The highest rates of inactivation occurred in the presence of the combination of Sodium benzoate and Potassium sorbate after 6 days, While LPS reduces E. coli O157:H7 2 log cfu/ml after 10 days. It seems necessary that the concerned authorities should impose regulation and bacteriological standards and take active part in the control of produced milk to ensure a maximum safety to the consumers.

Key words: *E. coli O157:H7, milk, sodium benzoate, LPS, potassium sorbate.*

INTRODUCTION

Milk is a highly perishable commodity and difficult to handle, especially in a country with high ambient summer temperature and lack of cooling methods.

E. coli O157:H7 constitutes a significance risk to human health world wide and the infection is associated with consumption of food of bovine origin (Philips *et al.* 2000), it causes acute renal failure in children (Fitzpatrick *et al.* 1991). The spectrum of clinical illness ranges from mild diarrhea, through bloody diarrhea and hemorrhagic uraemic

syndrome (HUS) and Thrombotic thrombocytopenic purpura (TTP) (Locking *et al.* 2001).

VTEC (verocytotoxin-producing Escherichia coli) O157:H7 has been identified as a possible contaminant of raw milk (Bryan, 1983). The gastro-intestinal tracts of ruminants, especially cattle, and humans are the main reservoirs of E. coli O157:H7 (Duffy *et al.*, (2001). Faecal contamination of raw cow's milk during its collection on dairy farms as well as milk filters are recognized as a major VTEC transmission route (Heuvelink *et al.*, 1998). Due to contamination of raw milk and/or improper processing, cow's milk has been implicated in foodborne outbreaks (Trevena *et al.*, 1996 and Keene *et al.*, 1997). In addition to foodborne transmission, E. coli O157:H7 may be spread by person to person as the source of infection is the feces of infected persons (principally sick persons, secondarily carriers) and objects contaminated by them (Griffn and Tauxe, 1991 and Dean-Nystrom *et al.*, 1997). E. coli O157:H7 constitutes 33% of milk borne general outbreaks of infectious intestinal diseases as a result of unpasteurized milk consumption (Gillespie *et al.*, 2003). Wachsmuth *et al.*, (1997) reported that raw milk was responsible for 5% of the outbreaks VTEC O157:H7 in the USA from 1982 to 1995.

Other verocytotoxin-producing serotypes (e.g. O26:H11, O103:H2, O113:H21) associated with human bloody diarrhea and HUS have also been isolated from sheep, calf and cattle faeces (Wells *et al.*, 1991).

Unhygienic conditions under which the animals are milked, small individual producer, long distance between the production and market areas, poor transportation, and insufficient or non-availability of milk cooling or chilling systems are the main problems of milk production. These problems lead to a considerable reduction in the shelf-life of milk consequently its souring (Jager, 1994 and Abd EL-Ghani and Sayed, 1997).

Preservatives are, generally, used to prevent or retard both chemical and biological deterioration of foods, to extend the shelf-life, retain nutritive value and to ensure the food safety by inhibiting or retarding the growth of pathogenic microorganisms. Addition of chemical preservatives to milk and milk products is widely used as an alternative method of cooling. Legal chemical preservatives are Sodium benzoate, Potassium sorbate and Lactoperoxidase system (Collins, 1971and Ismail, 1997). *The objective* of this study was to determine the prevalence of E. coli O157:H7 in raw milk and to evaluate the inhibitory

effect of sodium benzoate, potassium sorbate, combination of sodium benzoate and potassium sorbate, and activated Lactoperoxidase system (LPS) on the viability of E. coli O157:H7 in commercially sterile milk stored at 4 °C

MATERIAL and METHODS

I. Prevalence of Enterohemorrhagic Escherichia coli O157:H7 in raw milk

1. Collection and preparation of samples:
One hundred samples of raw milk were collected randomly from different shops, street-vendors and supermarkets in Alexandria and El-Behera Governorates, Egypt. Samples were transferred directly to the laboratory with a minimum of delay and were prepared according to APHA (1992).

2. Isolation and Identification of Escherichia coli O157:H7:

2.1. Enrichment technique: Milk samples were enriched in modified Tryptic Soya Broth (mTSB) supplemented with Novobiocin (20 mg/liter) and incubated at 37°C for 24 hours (DeBoor and Heuvelink, 2000).

2.2. Isolation on Sorbitol MacConkey agar: Loopfuls from the incubated broth were streaked onto Sorbitol MacConkey agar plates and incubated at 37°C for 24 hours (DeBoor and Heuvelink, 2000).

2.3. Identification of Escherichia coli O157:H7: Non Sorbitol fermenter colonies were identified biochemically as E. coli according to Varnam and Evans (1991) by the conventional IMViC, Urea hydrolysis and Triple sugar iron agar.

2.4. Identification of E. coli serogroup O157 (Oxoid,1996): Typical E. coli strains were screened by a rapid latex agglutination test (E. coli O157, Oxoid diagnostic reagent 620 M) for identification of E. coli serogroup O157.

2.5. Identification of H7 (Difco, 1996): Typical E. coli strains were screened by a tube agglutination test for identification of E. coli serogroup H7.

II. Effect of some chemical preservatives on the viability of Enterohemorrhagic Escherichia coli O157:H7 in milk

1. Effect of different concentrations of Potassium sorbate and Sodium benzoate on viability of E. coli O157:H7 in commercial sterilized milk stored at refrigeration temperature at (4±1 °C):

1.1. Organism: Culture of E. coli O157:H7 used in this study was obtained from the previously examined raw milk samples.

1.2. Experimental procedure:

1.2.1. Sterilized milk, which proved bacteriologically free from E. coli O157:H7, was inoculated with a suspension of 24 hours incubation E. coli O157:H7. The initial count was 1.8 $x10^3$ cells/ ml. Each inoculated milk sample was divided into 6 groups; Control (preservative free), A, B containing 0.05 and 0.1 % Potassium sorbate and C, D containing 0.05 and 0.1 % Sodium benzoate, respectively. While, group E contained a combination between Potassium Sorbate (0.1%) and Sodium benzoate (0.1%).

1.2.2. Effect of activated Lactoperoxidase system on viability of E. coli O157:H7 in thermalized milk stored at refrigerator temperature (4 ±1°C):

Fresh milk samples were heated at 63°C for 10 minutes (Thermization) in flasks immersed in thermostatically controlled water bath. The flasks were inoculated with a suspension, of 24 hours incubation, E. coli O157:H7. The initial count was 1.8 $x10^3$ cells/ ml. The inoculated milk sample was divided into 2 parts; one was kept as a control while the second was activated with LP-system by adding 14 mg of Sodium Thiocyanate (Merck, Germany) followed by 30 mg of Sodium percarbonate (Aldrich, USA) /liter of milk (IDF, 1988).

Control samples together with inoculated ones were kept at 4 ±1 °C for studying the survival of E. coli O157:H7 on Sorbitol MacConkey agar (APHA, 1992). Counts were determined at Zero time, then examined every day till eleven days storage at refrigerator temperature (4±1°C).

RESULTS

The obtained results are recorded in Table 1 and Figures 1-4.

DISCUSSION

I. Prevalence of Enterohemorrhagic Escherichia coli O157:H7 in raw milk

The result given in Table 1 revealed that out of 100 examined raw milk samples only one sample (1%) contained E. coli O157:H7.

This finding is in agreement with those reported by Steele *et al.*, (1997); Karakulska (2002); Murinda *et al.*,(2002) and Dontorou *et al.*, (2003).While higher incidences were reported by Clarke *et al.*,(1987)

and Awad (2002). On the other hand, Palmgren *et al.*, (1997), Aman *et al.*, (1998) , Massa *et al.*, (1999) and Abd EL-Hady *et al.*, (2003) could not detect *E. coli* O157:H7 in any of examined raw milk samples. Reported estimates on the prevalence of VTEC O157:H7 in raw cow's milk ranged from 0 to 10% (Hancock *et al.*, 1998 and Coia *et al.*, 2001). However, according to surveys carried out in various countries, for example Scotland (Coia *et al.*, 2001) the Netherlands (Heuvelink *et al.*, 1998) and Sweden (Jonsson *et al.*,2001), the prevalence of VTEC O157:H7 in raw cow's milk at farm level seems to be extremely low. Outbreaks of shiga toxin producing *E. coli* infection associated with serotypes O157:H7 or O157: H$^-$ had been documented at least in 14 countries on 6 continents (C.D.C., 1993; Liesegang *et al.*, 2000 and Allerberg *et al.*, 2001).

Although over 150 different OH serotypes of VTEC have been associated with human illness, the main serotypes implicated in hemorrhagic colitis, HUS and thrombotic thrombocytopenic purpura are *Escherichia coli* O157:H7 and *E. coli* O157:H$^-$ (Nelson *et al.*, 1998 and Vernozy-Rozand, 1999).

Nowadays verocytotoxin-producing Escherichia coli is considered an important emergent foodborne pathogen constituting a worldwide public health hazard either in the form of sporadic cases of infection or outbreaks (Vernozy-Rozand, 1999 and Leclercq *et al.*, 2001)

Moreover, the significant morbidity and mortality associated with VTEC infection largely as a result of hemolytic uremic syndrome (HUS) makes it a zoonotic problem of serious public health concern (Nelson *et al.*, 1998).

II. Effect of some chemical preservatives on the viability of Enterohemorrhagic Escherichia coli O157:H7 in milk.

a) Potassium sorbate

The results in Fig 1 reveal that a gradual decrease in numbers of E. coli O157:H7 occurred during the prolonged shelf-life of inoculated milk kept at 4±1°C. E. coli O157:H7 achieved a minimum count on the 7th and 10th day of storage before the samples spoiled at 8th and 11th day for samples containing 0.05 and 0.1% added Potassium sorbate, respectively. Potassium sorbate (0.1%) caused one log reduction in E. coli O157:H7 count by the end of the 10th day of storage. These findings agreed with those obtained by Narasimhan *et al.*, (1989) and El-Prince (1994) as they concluded that 0.1- 0.75% of Potassium sorbate inactivated E. coli. The inhibitory effect of Potassium sorbate on E. coli could be attributed to the reduction of intracellular pH of E. coli cells (Salmond *et al.*, 1984)

and the reduced uptake of required amino acids which are necessary for their growth (Ronning and Frank, 1987). Although the control samples were spoiled at 6th day as well as the treated samples, it is observed that Potassium sorbate greatly decrease the E. coli O157:H7 load in comparison with the control samples. E. coli O157:H7 survives or even multiplies in raw milk when stored at 8°C and reaffirm the need for pasteurization and holding the milk at < or= 5°C (Massa *et al.*, 1999 and Arias *et al.*, 2001).

b) Sodium benzoate

The data illustrated in Fig 2 verify that there was a steady decrease in numbers of E. coli O157:H7 during storage at refrigerator temperature (4±1°C) of inoculated milk samples containing 0.05% Sodium benzoate and reached a minimum count of $2.0x10^3$ at 8th before the samples being spoiled. While in milk samples containing 0.1% Sodium benzoate, E. coli O157:H7 sharply decreases in numbers from $1.8x10^3$ alls/ml to undetectable numbers by the end of the 8th day of storage.

c) Combination of Potassium sorbate (0.1%) and Sodium benzoate (0.1%)

It is evident from Fig 3 that E. coli O157:H7 was sharply decreased in inoculated milk during storage at (4±1°C). The combination could eliminate E.coli O157:H7 by the end of 6th day of storage. The obtained results verify that combination between Potassium sorbate (0.1%) and Sodium benzoate (0.1%) had a great inhibitory effect on E. coli O157:H7 in milk.

d) Effect of activated Lactoperoxidase system on viability of E. coli O157:H7 in thermalized milk.

The lactoperoxidase system (LPS) has been extensively investigated for dairy industry application (Farrag and Marth, 1992). When applied to dairy products, the major component of the system lactoperoxidase is present in the milk. The system is activated by the addition of thiocyanate and hydrogen peroxide in the form of sodium percarbonate. The activation of the LPS system in raw milk is used to prevent undue bacterial multiplication during collection and transport to the dairy processing plant in countries where refrigeration may not be feasible due to technical or economic reasons.

Fig 4 shows the inhibitory effect by the activation of LP-system on E. coliO157:H7 in milk stored at 4±1°C. LP-system causes a 2 log reduction within 10 days before the samples were spoiled. Similar trends were observed by swart *et al.*, (1990) who reported that activation of LP-system in raw milk stored at 5°C for 4 days decreases E. coli count. Also,

Girgis *et al.*, (1999) mentioned that, the activation of LP-system in cow's milk caused a sharp decrease in the total coliforms including E. coli after 2 hours. The action of LP-system may be explained on the basis of production of bacterial inhibitor hypothiocyanate (OSCN) which interfere with metabolism of bacteria with regard to oxygen uptake and lactic acid metabolism thereby inhibiting growth (Wlfson and summer, 1993 and De wit and Van Hooydonk, 1996).

It was concluded from the results obtained that combination of Potassium sorbate (0.1%) and Sodium benzoate (0.1%) has more inhibitory effect on E. coliO157:H7 so, it is recommended that this combination should be added to raw milk to improve its shelf-life. Measures for improved cleanliness of stables, food management, efficient sanitation and cleanliness of animals when transported; the hygienic production of milk and milk products; strict maintenance of the cold chain (processing and distribution); heat treatment; provision of information to food handlers and to consumers with special attention to groups at special risk; and consideration of decontamination procedures before consumption should be applied.

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Table 1: Prevalence of *Escherichia coli* O157:H7 and *Escherichia coli* O157:H$^-$ in raw milk samples.

	No. of samples	E. coli O157	*E. coli* O157:H7		*E. coli* O157:H$^-$	
			No.	%	No.	%
Raw milk	100	1	1	1	0	0

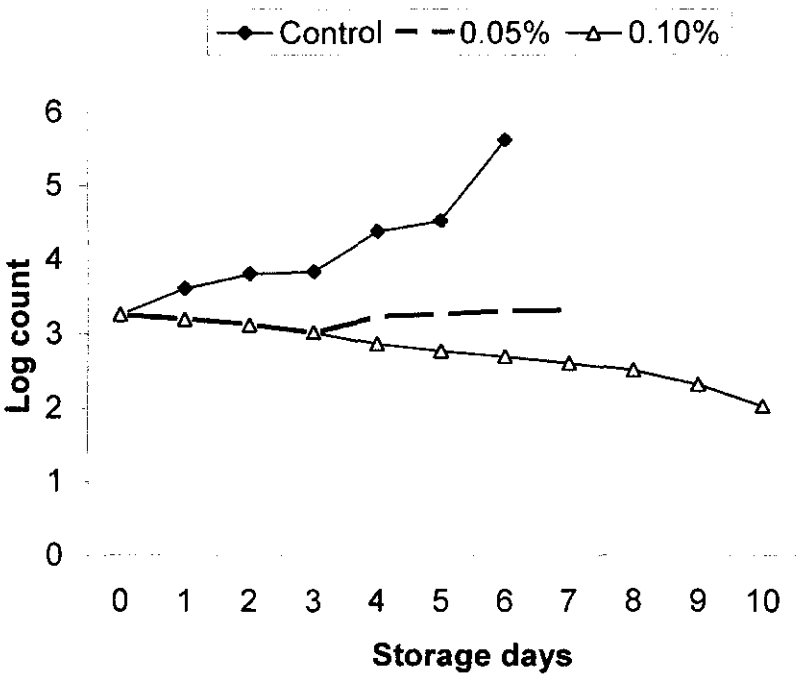

Figure 1: Effect of Potassium sorbate on viability of E. coli O157:H7 in sterile milk

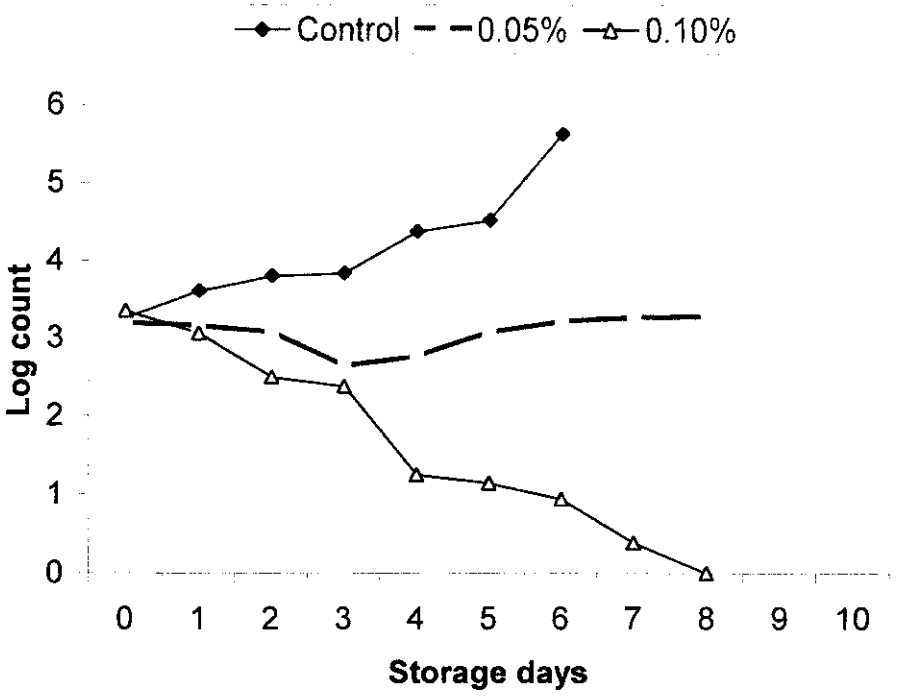

Figure 2: Effect of Sodium benzoate on viability of E. coli O157:H7 in sterile milk

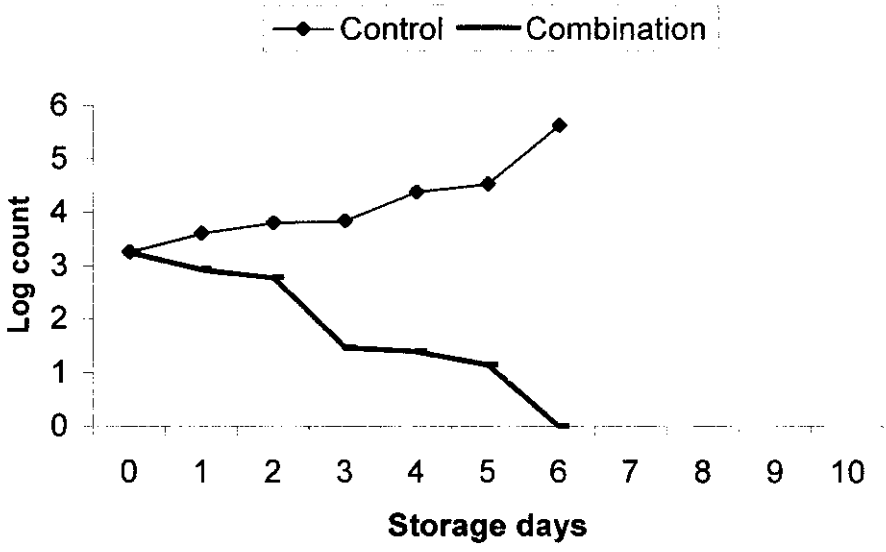

Figure 3: Effect of combination of Potassium sorbate (0.1%) and Sodium benzoate (0.1%) on viability of E. coli O157:H7

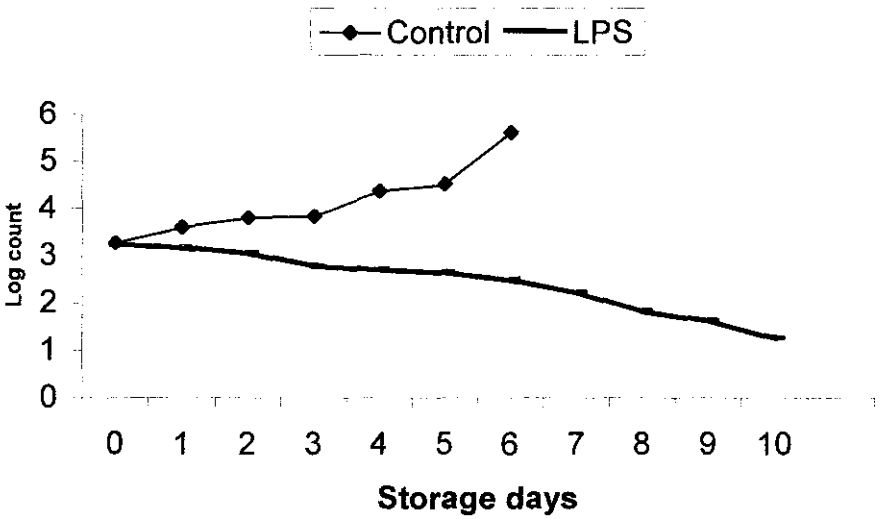

Figure 4: Effect of activated Lactoperoxidase system on viability of E. coli O157:H7 in thermalized milk.