EFFECT OF YOGHURT PROCESSING AND ICE CREAM MANUFACTURE ON VIABILITY OF SOME FOODBORNE BACTERIA

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ABSTRACT
Food borne bacterial gastrointestinal infections are important causes of morbidity and mortality worldwide, and despite successful control programs in some developed countries, theses infections continue to have a major impact on public health and economy. The objective of this study was planned to spotlight on the effect of yoghurt processing, ice cream manufacture and during storage period on viability of Enterohemorrhagic coli O157:H7 and Yersinia enterocolitica. Yoghurt was manufactured from raw milk, proved to be free from both organisms, in laboratory. Raw milk was inoculated with Enterohemorrhagic coli O157:H7 and Yersinia enterocolitica at density of 5.13×10^4 and 8.1×10^4, respectively and stored at 4°C. Samples of milk, curd and finished product were examined up to 12th day of storage for growth of both organisms as well as pH value. While, ice cream samples were inoculated at density of 1.55×10^5 and 8.2×10^5, respectively and stored at (-4°C) and (-18°C). The effect of freezing on growth and viability both organisms was examined daily up to 35th day of storage.
The growth pattern of Enterohemorrhagic coli during yoghurt processing and storage revealed that Enterohemorrhagic coli could survive for 9th day of storage before complete reduction occurred at 10th day. While, Yersinia enterocolitica could survive for 3rd day of storage before complete reduction occurred at 4th day. The survival characteristics of Enterohemorrhagic coli in ice cream revealed that freezing ice cream at (-4°C) and (-18°C) reduce Enterohemorrhagic coli count by 86.25 and 99.48%, respectively by the end of 35th day of storage. Yersinia enterocolitica reduced by 99.4 and 99.9 %, respectively by the end of 35th day of storage.
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. Moreover, enforcement of GMP and HACCP system inside dairy plants is of critical.

Key words: Enterohemorrhagic coli O157:H7, Yersinia enterocolitica, yoghurt, ice cream

INTRODUCTION
Milk is a highly perishable commodity and difficult to handle, especially in a country with high ambient summer temperature. Enterohemorrhagic coli O157:H7 constitutes a significance risk to human health worldwide and infection associated with consumption of food of bovine origin (Philips et al., 2000), and 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), thrombotic thrombocytopenic (TTP) and renal failure in children (Locking et al., 2001 and Razzaq VTEC (verocytotoxin–producing Escherichia coli) O157:H7 has been i as a possible contaminant of raw milk (Bryan, 1983). The gastro–intestin of ruminants, especially cattle, and humans are likely to present th reservoirs of E. coli O157:H7 (Duffy et al., 2001). E. coli O157:H7 co 33% of milk borne general outbreaks of infectious intestinal diseases as of unpasteurized milk consumption (Gillespie et al., 2003). While, Wachs al., (1997) reported that raw milk was responsible for 5% of the outbreak O157:H7 in the USA from 1982 to 1995.

Yersinia enterocolitica is a zoonotic, Gram-negative bacterium cap causing severe gastrointestinal infection (Varnam and Evans, 1991 an 1998). It produces a heat stable enterotoxin that is associated w poisoning strains in man (Bielecki, 2003). The frequent association organism to raw milk and its ability to grow in milk over a long period under freezing, thawing and constant freezing condition would facil survival in the environment and its transmission via milk (Larkin et al., 19 the importance of Yersinia enterocolitica as a cause of foodborne illnes between countries. In England and Wales, laboratory reports mostly cases increased from 45 in 1980 to more than 590 in 1989 (Adams an 2000). Unhygienic conditions under which the animals are milke individual producer, long distance between the production and marke poor transportation, and insufficient or non-availability of milk cooling, system are the main problems of milk production especially in de countries. These problems could be solved by rapid processing. The o of this study was planned to spotlight on the effect of yoghurt proces cream manufacture and during storage period on viability of Enterohem coli O157:H7 and Yersinia enterocolitica.

MATERIALS AND METHOD

I. Effect of yoghurt processing and storage on viability of E. coli O and yersinia enterocolitica:

(a) Test organisms

1. Enterohemorrahgic E. coli (E. coli O157:H7):

Escherichia coli O157: H7 strain was kindly obtained from Depart Microbiology, Faculty of Veterinary Medicine, Giza, Egypt. The inocu growth study was prepared by streaking E. coli O157: H7 from refrigerat agar slant culture into Tellurite Cefixime Sorbitol–MacConkey aga (TCSMAC) (Difco, Detroit, USA). Plates were incubated at 37°C for 24 h separate colony was then picked and inoculated into sterile modified tr broth (TSB) (Difco, Detroit, USA). Broth tubes were incubated at 37°C fo After two successive transfers and incubation, the culture was maint sterile 0.1% peptone water which served as the working culture.

2. Yersinia enterocolitica:

Yersinia enterocolitica, strain was kindly obtained from Departr Microbiology, Animal Health Research Institute, Dokki, Giza, Egypt, wa in trypticase soy broth at 22 °C for 18 hrs. After two successive trans incubation, the culture was maintained in sterile 0.1% peptone wat served as the working culture.

(b) Raw milk used for yoghurt manufacture:
Raw milk was taken from the experimental station of the Department of Animal Production, Faculty of agriculture, Alexandria University to be used for yoghurt manufacture in the laboratory. The milk was dispatched to the laboratory in clean, dry and sterile flasks with a minimum of delay.

(c) Yoghurt cultures:
Yoghurt cultures (IST from 2% NIZO) were obtained from Department of Milk and Dairy Technology, Faculty of Agriculture, Alexandria University. The cultures were thawed at room temperature (20 °C). Two consecutive transfers in sterile skim milk were made and incubated at 37 °C for 24 hours prior to use in yoghurt manufacture.

(d) Preparation of yoghurt:
Yoghurt was manufactured in the laboratory. Raw milk was heated to 90 °C for 30 minutes and then cooled to about 40 °C. The starter cultures (2%) of Lactobacillus bulgaricus and Streptococcus thermophilus in a ratio of 1: 1 were added to milk and thoroughly mixed. The prepared cultured milk was divided into two parts. To first one E. coli O157:H7 was added to milk to provide the desired number of pathogen per ml ($5.13x\ 10^4$) and to the other one Yersinia enterocolitica was added to milk to provide the desired number of pathogen per ml (8.1×10^4). The samples were incubated at 45 °C to be coagulated. Samples of milk, curd and finished product stored in refrigerator temperature (4 ± 1°C) were examined up to 12 days of storage for growth of E. coli O157:H7 and Yersinia enterocolitica count/g.

II- Effect of freezing on viability of E. coli O157:H7 and yersinia enterocolitica during storage of ice cream (– 4 and –18 °C):

(a) Collection of ice cream powder samples:
Packets of ice cream powder were purchased from various supermarkets at Alexandria Governorate and dispatched to the laboratory.

(b) Preparation of ice cream samples:
Two ice cream samples were prepared in the laboratory according to the manufacturer (Egyptian Dairy & Food Company). One was inoculated with prepared culture of E. coli O157:H7 to obtain a count of $1.55x10^6$. The second was inoculated with prepared culture of yersinia enterocolitica to obtain a count of 8.2×10^6. Each sample was divided into two portions, the first was kept at freezing temperature (– 4 °C) and the other was stored at deep freezing temperature (–18 °C). The effect of freezing on the growth and survival of E. coli O157:H7 and yersinia enterocolitica was determined daily up to 35 day of storage.

III. Enumeration of E. coli O157: H7:
E. coli O157: H7 count was achieved by surface direct plating of decimal dilutions of prepared samples **(APHA, 1992)** in which 0.1 ml of each serial dilution was surface plated into Tellurite Cefixime Sorbitol–MacConkey agar plates (TCSMAC) **(Oxoid, 1998)** and incubated at 37°C for 24 hrs. Typical E. coli O157: H7 colonies are neutral, gray with a smoky center and 1–2 mm in diameter was counted.

IV. Enumeration of yersinia enterocolitica:
Yersinia enterocolitica count was achieved by surface direct plating of decimal dilutions of prepared samples **(APHA, 1992)** in which 0.1 ml of each serial

dilution was surface plated into the surface of the selective medium, Cefs Igrasan–Novobiocin (CIN) **(Oxoid, 1998)** and incubated at 22 °C for Typical colonies of yersinia enterocolitica are deep red center with a rathe border and translucent outer zone were counted.

RESULTS

The obtained results illustrated in figures 1, 2, 3 and 4.

DISCUSSION

1. Effect of yoghurt processing and storage on viability of E. coli O15

Figure (1) revealed the population of E. coli O157:H7 changed with c rates during the manufacturing and refrigerated storage of yoghurt. Fr initial milk inoculation until clotting (Zero time), the inoculum levels of O157:H7 increased from 5.13x104 ± 3.21 x103 to 2.13x105 ± 5.0 x1C means that, bacterial cell number increased during yoghurt manufactu nearly 10–fold (1 log cycle) as a result of physical entrapment in the c addition to this, growth of E. coli O157:H7 may also occur during manufacturing as the temperature used for yoghurt incubation is appr the optimum growth temperature for that organism. It is well known that c strains of E. coli O157 exhibit slightly different growth temperature optir 38.5 to 42.5 °C (Gonthier et al., 2001). After two days of refrigerated sto coli O157:H7 count begins to significantly decrease to 1.65x105 ± 3.5 which accompanies the fall down of yoghurt pH to 4.5. The decline o population of E. coli O157: H7 after 48 hrs of fermentation may b attributed to the production of bacteriocins, hydrogen peroxide and eth starter cultures (Frank and Marth, 1988). Comparatively higher reductior E. coli O157: H7 was recorded just after formation of the curd by Dinee (1998). Such results differences could be attributed to using of differe inoculum sizes upon processing of yoghurt and/or variability in the v among the tested strains (Oksuz et al., 2004).While, the number of O157:H7 declines continuously during refrigerated storage of yoghurt, sig numbers may still exist in the yoghurt after 9 days 4.80x102 ± 2.50 x10 of great concern considering that expiry date of yoghurt is typically set to 15 days of manufacturing. These results also indicate that yoghurt ma milk contaminated with E. coli O157:H7 at level of 5.13x104 ± 3.21 x103 to contain the bacterium at levels that are known to cause illness by th reaches the consumer. This of concern to both yoghurt manufactur consumers because of the low infectious dose associated with E. coli C infections (Doyle et al., 1997). The ability of E. coli O157:H7 to in Adaptive Tolerance Response (ATR) when exposed to mild acid co confers a higher resistance on subsequent exposure to strong acid co (Doyle et al., 1997 and Jordan et al., 1999). The induction of an ATR acid conditions in the yoghurt may promote greater resistance to aci passage through the stomach, thereby low ingested number of E. coli C can cause infection. Moreover, it has been shown that casein of dairy protects pathogens from acidic stress (Rubin, 1985). This may be anoth that enables E. coli O157:H7 to survive in the acidic conditions of yoghu milk fermentation produce anaerobic conditions within the ferment

products, it is thought that anaerobic growth of E. coli O157: H7 in an acidi medium, like yoghurt, results in the development of acid tolerance (Cheng an Kaspar, 1998). The development of similar acid tolerance effects would b expected to also occur in this study. It seems that the presence of E. coli O157 H7 and its survival at both low temperature and pH in this study confirmed th implication of acidic food in some recent outbreaks due to EHEC infectio (Sharpe et al., 1995).

2. **Effect of yoghurt processing and storage on viability of Yersini enterocolitica:**

Figure (3) revealed that Yersinia enterocolitica was slightly decreased in count from 8.1 x 104 ± 5.0 x 102 to 4.4 x 103 ± 1.5 x 102 cfu/g this may be due to th effect of the processing as well as the decrease pH value from 6.4 to 4.7 Yersinia enterocolitica remained viable for 3 days and during the same perio pH reduced to 4.2. Nearly similar results were reported by Halawa (1995) wh studied the effect of yoghurt processing and cold storage temperature o survival of Yersinia enterocolitica using reference strain (ATCC 27729) whic was inoculated into milk in low and high concentration (103, 107 cfu/ml) befor processing of yoghurt then stored at 4°C. He found that low and high coun slightly reduced due to increase the acidity percent while in the stored product a 4°C, Yersinia enterocolitica reduced to 4.8 x 102 starting from 32 x 103 after 1 hr. while, Canganella et al., (1998) investigated the survival of Yersin enterocolitica in fruit yoghurt after inoculation at two different levels (102–10 cfu/ml and 104–106 cfu/ml) during storage at 4 °C. The survival was n significant (3 days) but did not be detected after 2 weeks except when the siz of the initial inoculum was larger than 105 cfu\ml in this case viable cells of th pathogen were still recovered after 17 days of storage and they concluded th survival of Yersinia enterocolitica was better during storage at 4 °C than 8 °(Longer persistence for viable organism was recorded by Abd El-Hady (199 who reported that Yersinia enterocolitica could survive for 7 days in yoghurt.

3. Effect of freezing on viability of E. coli O157:H7 in ice cream

Freezing has been established as an excellent method of preserving quality foods. It preserves the taste, texture and nutritional value of foods better tha any other method and as a result extensive quantities of foods are now froze worldwide (Marilyn and Yen–con, 1997).

Results recorded in Figure (2) revealed the growth pattern of E. coli O157:H during frozen storage of ice cream at – 4 and –18 °C. The initial population 1.5 x 10^5 ± 3.15 x 10^4 decreased gradually to reach 2.13 x 10^4± 8.21 x 10^3 and 7.9 x 10^3 ± 1.32 x 10^2 with reduction percent of 86.25 and 99.48, respectively by th end of 35th day of storage. Same findings were recorded by Susan and Camer (1994) and Abou–Zeid et al., (2001) who reported that E. coli O157:H7 prov capability to survive very well in refrigerated dairy products. Although, Wang al., (1997) noted that E. coli O157:H7 did not grow at 5oC in milk and t population decreased. Although, ice cream has not yet been directly implicat in outbreaks of E. coli O157:H7 (Rothwell, 1990), the organisms can survive w at–20oC and at–18oC for up to 9 months (Doyle and Schoeni, 1984).

4. Effect of freezing on viability of Yersinia enterocolitica in ice cream

As observed in Figure (4) Yersinia enterocolitica counts in ice cream samp that there was a gradual reduction in counts from $8.2 \times 10^5 \pm 5.0 \times 10^4$ cfu/g $4.3 \times 10^3 \pm 5.0 \times 10^2$ cfu/g by the end of the 35th day of storage at freezi temperature (- 4 °C) with reduction percent of 99.4. While in case of ice cre samples stored at (-18) reached a count of $5.0 \times 10^2 \pm 1.2 \times 10^2$ cfu/g by the e of the 35th day of storage with a reduction percent of 99.9. Yersinia enterocolit could withstand freezing and surviving for long periods in frozen food, even af repeated freezing and thawing (Toora, 1992). These result substantiated Annamalai and Venkitanarayanam (2005) who reported that Yersi enterocolitica is a foodborne pathogens that had been implicated in outbreaks foodborne illness involving cold stored foods. From the previous findings, it co be concluded that the storage temperature has a great influence upon Yersi enterocolitica as storage at – 18 °C is considered the effective way to get ride Yersinia enterocolitica.

Measures for improved food management, efficient sanitation and cleanliness animals when transported; the hygienic production of milk and milk produc strict maintenance of the cold chain (processing and distribution); he treatment; provision of information to food handlers and to consumers w special attention to groups at special risk; and consideration of decontaminati procedures before consumption should be applied.

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الملخص العربي

ير تصنيع الزبادي و الأيس كريم على حيوية بعض البكتيريا الممرضة

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بر الزبادي و الأيس كريم من منتجات الألبان واسعة الانتشارلما تحتويه من عناصر غذائية ة تجعلهم ذوى قيمة عالية إلا أنهم قد يتعرضوا للتلوث من مصادر مختلفة أثناء الإنتاج أو داول مما يؤثر على سلامتهم نتيجة تغيرات غير مرغوبة تجعله غير صالح للاستهلاك دمي. أجريت هذه الدراسة لمعرفة تأثير تصنيع الزبادي و الأيس كريم علي حيوية ميكروب يشريكية القولونية عترة O157:H7 المسببة للالتهاب المعوي النازف و ميكروب اليارسينيا روكوليتيكا. وقد تم تصنيع الزبادي من لبن خام خالي من الميكروبات المراد دراستها و تم ن اللبن بعدد معلوم من ميكروب الايشريكية القولونية عترة O157:H7 المسببة للالتهاب عوي النازف و ميكروب اليارسينيا انتيروكوليتيكا و تم تخزينه عند درجة حرارة الثلاجة ٤ و قد تم أخذ عينات من اللبن الخام بعد الحقن و الخثرة المتكونة و الزبادي حتى اليوم الثاني ر من التخزين و كذلك قياس الأس الهيدروجيني. و كذلك تصنيع الأيس كريم و حقنه يكروبات ذاتها و تخزينه عند درجة حرارة -٤ و -١٨ م°. وقد اسفرت النتائج ميكروب أن يشريكية القولونية عترة O157:H7 لها القدرة علي المقاومة في عينات الزبادي حتى اليوم سع قبل أن يتم القضاء على الميكروب بعد عشرة أيام من التخزين في درجة حرارة لاجة. بينما ميكروب اليارسينيا انتيروكوليتيكا له القدرة على الحياة حتى اليوم الثالث من خزين. ووجد أن تصنيع الأيس كريم يقلل من عدد الايشريكية القولونية عترة O157:H7 بة 86.25 و 99.48% المخزن عند درجة حرارة -٤ و -١٨ م° بينما ميكروب اليارسينيا روكوليتيكا يقل بنسبة 99.47 و 99.93% المخزن عند درجة حرارة -٤ و -١٨ م° . و تم مناقشة الأهمية الصحية لهذه الميكروبات و الإحتياجات و العليير الواجب توافرها حتى يتم حكم في مصادر التلوث.