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Assessment of the Growth and Survival of *Escherichia coli* O157:H7 During the Manufacture and Storage of Iranian White Cheese and Probiotic Cheese.

[1]Hiva karimi darehabi and [2]Parang Nikmaram

[1]Department of food Hygiene, sanandaj Branch, Islamic Azad University, sanandaj, Iran
[2]Department of Food Science, University of Tehran, Karaj, Iran

Abstract: During the past two decades probiotic (health promoting) micro-organisms have been increasingly included in various types of food products, especially in fermented milks. *E. coli* O157:H7 outbreaks associated with consumption of ovine or cow cheese products have been reported in several countries. The aim of the present study was to determine the effect of pH and different probiotic bacteria on survival of *E. coli* O157:H7 during manufacture and storage of feta cheese and probiotic cheese. Cheese was manufactured using pasteurized cow milk and inoculated with *E. coli* O157:H7 with inoculums level of 10^4 cfu/ml. Two batches of cheese were prepared, one of them was treated with starter while the other sample use mix starter and probiotic bacteria (*Lactobacillus acidophilus* 4962, *Lactobacillus acidophilus* La5). Cheese samples were analyzed for *E. coli* O157:H7 during manufacture and storage period. During cheese manufacture the umber of *E. coli* O157:H7 increased by 10^7cfu/g, but during ripening and cheese storage the number of organism decreased significantly in the cheese samples made with probiotic bacteria than feta cheese ($P<0.05$).results presented in this study showed that the manufacturing procedure of feta cheese in brine and probiotic cheese do not eliminate *E. coli* O157:H7, although the population of the organism decrees in probiotic cheese than Iranian white cheese cheese.

Key words: Probiotic cheese · Iranian white cheese · Escherichia coli O157:H7

INTRODUCTION

Escherichia coli O157 is a bacterium that commonly lives in the intestines of people or animals. It is a Gram-negative, facultative anaerobic and nonsporulating organism [1]. It is a newly recognized bacterial zoonosis that originates from the gut of infected cattle and is an enterohemorrhagic strain of the bacterium *E. coli* and a cause of foodborne illness. Infection often leads to hemorrhagic diarrhea and occasionally to kidney failure, especially in young children and elderly. Transmission is via the fecal-oral route and most illness has been associated with eating undercooked, contaminated ground beef, swimming in or drinking contaminated water and eating contaminated vegetables. The organismis known to produce one or more Shiga toxins, which may produce diarrhea, hemorrhagic colitis and life-threatening hemolytic uremic syndromein humans and animals [2,3]. The organism is destroyed in pasteurization process, but insufficient heat treatment of ground meat and raw milk forms a potential infection risk [4].

It has been documented that probiotics have been used as food supplements to prevent diseases and improve the health of both human beings and animals. Probiotics act differently from antibiotics, probiotics are living micro-organisms that help the useful existed micro-organisms to grow in the gut and thus maintain the of their hosts [5]. This useful effect can theoretically arise from one of the following mechanisms: 1-Weakening reactions which cause toxic and carcinogenic metabolites. 2 - Enhancing enzymatic reactions involved in detoxifying potentially toxic materials which either have been swallowed or produced by the body. 3 -Inducing enzymes in primates to digest complicated foods or helping the body provide enzymes through bacteria. 4 - Producing vitamins and other necessary nutrients which are not adequately found in the food basket [6,7]. In Iran, similar to other countries, a large amount of traditional cheeses are manufactured from raw milk and consumed freshly or after ripening in salt brine. Cheese made with unpasteurised milk is a potential vehicle for transmission of *E. coli* O157 to the consumer [8,9]. The objectives of

Corresponding Author: Hiva karimi darehabi, Department of food Hygiene, sanandaj Branch, Islamic Azad University, sanandaj , Iran, E-mail: hiva60iran@yahoo.com.

this study were thus to incorporate the selected probiotic strains into Iranian white cheese and examine the performance of these organisms in terms of survival of *Escherichia coli* O157:H7 during the manufacture and storage of Iranian white cheese and probiotic cheese.

MATERIALS AND METHODS

Milk: Milk supply of exceptionally favorable bacteriological and chemical quality is essential for the production of cheese of consistently good quality.To perform the required testing Pasteurized cow milk was obtained from Iranian Dairy Industries Co. and stored at 4°C. The quality of the milk was within the limits specified in the current Iranian standard for production of cheese (Fat = 2.5%, SNF = 8.9% and pH = 6.7) [10]. It was evaluated for the lack of antibiotic residues (copan test).

Bacteria Strain: Probiotic cheese prepared from, *Lactobacillus acidophilus* La5 and *Lactobacillus acidophilus* 4962 were obtain from Chr. Hansen's laboratory (CH-1, Denmark) . These strains were selected because they are commercially available cultures having documented health benefits. The strains were activated by growing at least two times at 30°C overnight in 12% (w/v) sterile reconstituted skim milk (RSM) containing 2% (w/v) glucose and 1.2% (w/v) yeast extract, prior to inoculation (2%, v/v) of the bulk culture in the same medium. Toxigenic strain of *E. coli* O157:H7 (ATCC 25922) was obtained from institute Veterinary Medicine, University of Tehran. This strain was activated during two consecutive cultures in 50 ml brain-heart infusion (BHI) broth for 18-20 h until the optical density reached 0.8 to 0.9 at 600 nm, which corresponded to approximately 1×10^9 cfu/ml. The culture was diluted to obtain a concentration of 10^7 cfu/ml. One ml of this culture was added to 10 L of milk to obtain a 10^4 cfu/ml.

Starter Production: Lyophilized direct vat type thermophilic yoghurt culture containing *Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus* (Chr. Hansen's laboratory, FD-DVS CH-1, Denmark) was used to make the Iranian traditional white brined cheese.

Procedure of Making Iranian White Cheese: To evaluate the effect of probiotic strains on growth and metabolism *E. coli* O157: H7, two baches were prepared: first Iranian white cheeseprepared, one of them was treated with 0.2 U/L starter (at 35°C) and 10^4 cfu/ml *E. coli* O157:H7 while the other sample was treated with starter, probiotic strains and 10^4 cfu/ml *E. coli* O157:H7. To speed up the clotting or reducing the amount of rennet needed, CaCl2 (0.02% w/v) was added. Rennet (Chr.Hansen's Laboratory, HANILASE L 3500) was then added to achieve the final concentration of 0.002% (w/v). Cheese was maintained at 35°C for 1 h to curdle. The curd was cut into 2 × 2 × 2 cm3 and allowed to drain. The mixture was agitated and drained for 1 h. Following drainage, the curd was placed in stainless steel press for 6 h, to fuse the curd grains into a continuous mass (7 h). The moulded cheese was cut into 7 × 7× 7 cm^3 pieces and kept immersed in 20% solution of pasteurized salt brine for 8 h at 18°C (15 h). After salting, cheese pieces were aseptically packed in 6% salt brine and hold at 14°C to ripen. The specimens were then kept at 4°C [11]. During ripening and storage period, the samples were analysed on dogs 15, 30, 45 and 60.

Microbiologic Studies: For detection of *E. coli* O157:H7, 25 g of shredded lettuce were added to 225 ml of modified Trypticase soy broth (m-TSB) (Merck) and incubated overnight at 37°C. The enrichment broth was streak-cultured on Sorbitol MacConkey agar (HIMEDIA M298, India) surfaces containing cefixime (0.05 mg/l), potassium tellurite (2.5 mg/l) and then incubated overnight at 37°C [12]Nonsorbitol-fermenting colonies on CT-SMAC were counted and 5-10 colonies were chosen to confirm by latex-agglutination with the *E. coli* O157 latex kit (Bahar afshan). Latex agglutinating isolates were further confirmed biochemically in SIM, MR-VP broth, Simon's citrate agar and TSI agar. *E. coli* O157:H7 are glucose, indole and methyl red positive, but negative for lactose, sucrose, Voges-Proskauer, citrate, CO2 and SH2. Then biochemically confirmed *E. coli* O157:H7 colonies were counted.

Chemical Analysis: The pH of cheese was measured using a pH meter (Testo 230) with a glass .Moisture content was,total solid and Salt contend, [13].Triplicate tests were performed for each analysis.

Statistical Analysis: The microbiological data were analysised using the general linear model procedure (SAS, 1992). Analysis of the variance followed by Duncan's multiple range test was employed to find significant differences ($P<0.05$) between the treatments.

RESULT AND DISCUSSION

E. coli O157:H7 is a bacterium that causes foodborne illness. Symptoms of *E. coli* infection include abdominal cramping and diarrhea, which can become bloody [14,15].

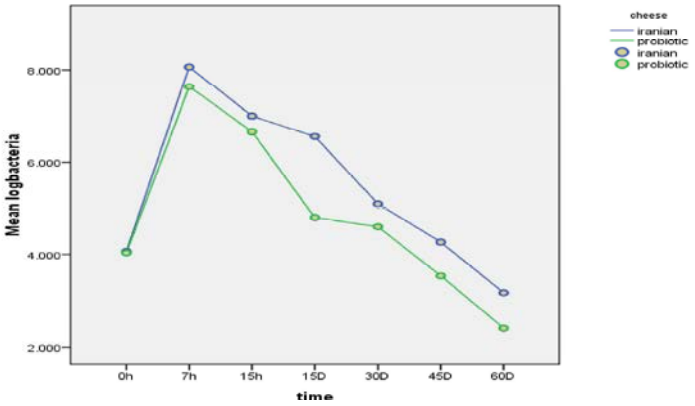

Fig. 1: *Escherichia coli* O157:H7 counts during the manufacture and storage of of Iranian white cheese and probiotic cheese

Table 1: Changes in mean of NaCl content, pH and E. coli O157:H7 counts in cheese samples made with starter and without starter

		Nacl		Hp		Log Bacteria	
Cheese type	Time	Mean	Std.Deviatio	Mean	Std.Deviation	Mean	Std.Deviation
	0 h	--	--	6.6333	0.05774	4.007	0.0077
	7 h	--	--	5.9	0.1	8.067	0.0528
Iranian	15 h	3.7232	0.15275	5.6333	0.05774	7.1	0.1
	15 d	3.9872	0.30551	5.4	0.1	6.567	0.1528
	30 d	4.0667	0.20817	4.9	0.1	5.1	0.1
	45 d	3.8	0.26458	4.6667	0.11547	4.267	0.2082
	60 d	3.7	0.1	4.4067	0.00774	3.167	0.1528
	0 h	--	--	6.6333	0.05774	4.007	0.0077
	7 h	--	--	5.6333	0.15774	7.633	0.0577
	15 h	3.9333	0.15275	5.3667	0.11547	6.667	0.2082
	15 d	4.1667	0.23094	5	0.1	0.48	0.1732
	30 d	3.9	0.17321	4.8	0.1	4.6	0.1
Probiotic	45 d	3.8	0.2	4.4333	0.15275	3.533	0.3512
	60 d	3.55	0.1	4.2667	0.05774	2.4	0.1

In severe cases, *E. coli* can cause kidney failure or even death [16]. The physicochemical properties and counts of *E. coli* O157:H7 in milk and cheeses made with probiotic and without probiotic strains culture, during manufacture, ripening and storage are given in table 1. *E. coli* O157:H7 was not isolated from the samples of pasteurised milk, starter culture, rennet, CaCl2 or salt brine. The counts of *E. coli* O157:H7 in all of the cheeses increased continuously from the initial inoculum level by about 3 logs in 7 h during manufacture. At the end of 15 h, the NaCl concentration in the cheese was 3.8%. During ripening, in the cheeses made with probiotic strains, the pathogen population decreased significantly ($P<0.05$) to 5 log cfu/g, whereas they remained relatively stable (about 10^7 cfu/g) in the cheeses made without probiotic starains. At those storage times, the pH was dropped to 4.7 and 5.1 in the cheese samples with and without probiotic strains, respectively. The pH of the cheeses made with probiotic dropped gradually to 4.3 on day 60.

At the end of the storage time, survival of *E. coli* was significantly lower ($P<0.05$) in cheese with probiotic compared to that without probiotlc strains (Fig. 1) that indicates antagonistic activity against E. coli O157:H7.

E. coli O157:H7 is the most important enterohemorrhagic (EHEC) serotype (the type of E. coli bacteria) associated with human disease occur worldwide; Other EHEC are probably also widely distributed [17]. The importance of some serotypes may vary with the geographic area, but other serotypes of EHEC strains are emerging as important pathogens throughout the world. Among them are E. coli O26:H11 and O111: NM, which are pathogens for humans and young calves [15,18]. With probiotic, E. coli bacteria substantially reduced fecal shedding of E. coli[11]. Use of colicinogenic *E. coli* strains and probiotic bacteria has been demonstrated to prevent *E. coli* O157:H7 faecal shedding or prevalence in cattle [19, 20].

Probiotic activity was largely inhibitory since the probiotics bacteria can reduce the level of *E. coli* O157 carriage and faecal shedding in cattle and calves [21] and decreased the severity and duration of diarrhea in *Escherichia coli* O157:H7-infected infant rabbits [22].Probiotic was reduced gastric inflammation and bacterial colonization in Helicobacter pylori-infected animals [23].

Inhibition of E. coli O157:H7 by *Lactobacillus plantarum* strain 299 v to HT-29 cellsusing lower *E. coli* O157:H7 counts (10^5 CFU/well) and slightly higher counts of tested probiotic strain (10^7 to 10^9 CFU/well). In this study, use of *Lactobacillus acidophilus* La5 and *Lactobacillus acidophilus* 4962 decreasd the grouth and metabolism *E. coli* O157:H7 and count of that (10^4 to 10^2 CFU/ml) [23].

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