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BEHAVIOR OF *Escherichia coli* O157:H7 DURING THE RIPENING OF HERBY CHEESE MANUFACTURED FROM RAW MILK

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Abstract:

This study was conducted to determine the survival of *Escherichia coli* O157:H7 during the ripening period of herby cheese made traditionally from raw cow milk. The cheese milk was inoculated with *E. coli* O157:H7 at the level of 3 log and 5 log cfu/mL, and then both manufactured herby cheeses were divided into two groups equally. The herby cheeses were stored by using two different methods for ripening, either embedding into the soil or putting into brine, and analyzed on day 1, 7, 15, 30, 60 and 90 of ripening. At the end of the storage period, *E. coli* O157:H7 could not be detected in embedded herby cheese at both levels of the inoculation; whereas the number of the bacterium was just decreased to 2.30 and 4.48 log MPN/g in brined herby cheese for each inoculation levels respectively. Additionally, micrococci/staphylococci count, acidity and salt values in all cheese groups were higher compared than the initial level; total mesophilic bacteria, lactic acid bacteria, enterobacteriaceae count and a_w value were lower than the initial level. While pH value was higher in embedded cheese than initial level, it was lower in brined cheese. In conclusion, *E. coli* O157:H7 could survive at least 60 days in embedded herby cheese and till the last days of the ripening in brined herby cheese. This point should be taken into account for the potential risk to public health.

Keywords: *E. coli* O157:H7, Herby cheese, Traditional, Growth, Survival

Introduction

Escherichia coli O157:H7 is a major foodborne infectious pathogen that causes hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and can lead to death (Doyle and Cliver, 1990). Many outbreaks and sporadic cases of foodborne illness caused by *E. coli* O157:H7 have been reported since the first one was recognized in 1982. In recent years, the incidence of the disease associated with this organism has steadily increased worldwide. *E. coli* O157:H7 is currently the most frequently isolated serotype in North America, and outbreaks have occurred with the highest incidences in Scotland, Canada, Japan, and the United States (Griffin and Tauxe, 1998; Parry and Palmer, 2000).

E. coli O157:H7 can survive at low temperatures as well as under acidic conditions, and the infectious dose is relatively small (Coia, 1998; Park et al., 1999). Due to the direct and indirect link to bovine products in outbreaks, dairy cattle have been implicated as the primary reservoir of this organism. The principal foods linked to transmission of the organism have been ground beef and raw milk (Doyle and Cliver, 1990). Cheese contamination with *E. coli* O157:H7 may result from inadequate pasteurization of raw milk or postpasteurization contamination. Currently, traditional cheese manufactured from unpasteurized milk has been consumed in many parts of the world. Many outbreaks of foodborne diseases due to cheese have been reported since 1983 (IFST, 1998). The isolation of *E. coli* O157:H7 was reported in 1-6.6 % of various native cheeses in Turkey (Aksu et al., 1999; Aslantaş and Yıldız, 2002).

Studies on cheese made from *E. coli* O157:H7 inoculated milk indicated that the pathogen could survive or even grow in different types of cheese, depending on the conditions during the manufacture and ripening of cheese. Survival of *E. coli* O157:H7 was determined during the manufacture and storage of Camembert and Feta cheeses at 2 °C for 65 and 75 days, respectively (Ramsaran et al., 1998). Reitsma and Henning (1996) found that the pathogen survived during the manufacture and curing of Cheddar cheese for 158 days. In another study, Özer et al. (2004) reported that the counts of *E. coli* O157:H7 decreased to zero in scalded Urfa cheese after 30 days, whereas this organism survived up to 90 days in unscalded Urfa cheese. Additionally, some investigators demonstrated that *E. coli* O157:H7 was capable of survival in Turkish white brined cheese (Küplülü et al., 1999) and smear-ripened cheese (Maher et al., 2001) for at least 90 days.

Herby cheese, called “Otlu peynir” in Turkish, is mainly produced in eastern and southeastern parts of Turkey. Traditionally herby cheese is manufactured from raw sheep’s and cow’s milk, and some herbs are added to the curd. After this procedure, the fresh cheese is ripened at storage for 3 months. In industry, production of herby cheese has modified the process and the only difference between the industrial production and the traditional one is the use of pasteurized milk instead of raw milk (Tunçtürk and Coşkun, 2002; Sağdıç et al., 2003).

There are many studies regarding the microbiological, chemical and sensorial properties of herby cheese. But, the behavior of *E. coli* O157:H7 in herby cheese is not known. Therefore, the purpose of the work was to evaluate the potential growth and survival of *E. coli* O157:H7 during the different ripening and storage of herby cheese.

Materials and Methods

Samples

For cheesemaking, 160 liters of raw cow milk obtained from Dönerdere Agricultural Development Cooperation (DÖN-KOOP, Van, Turkey) was used. *Escherichia coli* O157:H7 KUEN 1461 strain, obtained from Culture Collections of Industrial Microbiology and Biotechnology Association (KÜKEM, İstanbul, Turkey) was used to inoculate raw milk for the experimental herby cheese production. Commercial liquid rennet (Mayasan®, İstanbul, Turkey) with clotting activity of 1/16000 was used in the study. The herb locally known as Sirmo (*Allium* spp.) and available in brine at retail outlets was used for the cheese production.

Preparing Inoculum

E. coli O157:H7 strain was transferred into Tryptic Soy (CASO) Agar (TSA, Merck, Darmstadt, Germany) and incubated at 37 °C for 24 h. Then the tubes that contain 10 mL of Brain Heart Infusion Broth (Oxoid, Hampshire, UK) were inoculated by taking one colony from the strain and the tubes were incubated at 37°C for 24 h. Following the centrifuge process, the supernatant was removed and then 10 mL of sterile saline was added to the pellet. The sediment was mixed in vortex until it was homogenized, and the remaining su-

pernatant was removed by a subsequent centrifugation. After diluting the pellet with 10 mL of sterile saline, the initial solution was acquired. Decimal dilutions were prepared with the acquired solution. The turbidity levels of dilutions were measured with a spectrophotometer (Minufuge RF Heraeus Sepatech, Berlin, Germany) at 578 nm wavelength. At the same time, the dilutions were inoculated into TSA and the number of microorganisms and the turbidity levels measured in spectrophotometer were compared. The required amount to contaminate the milk at the level of log 3 and log 5 cfu/mL was obtained from the acquired dilutions.

Cheesemaking

Depending on the inoculation levels of *E. coli* O157:H7 and the cheese preservation methods, 4 types of cheese were produced. Cheese milk was contaminated with *E. coli* O157:H7 at log 3 cfu/mL in GC (cheese milk was inoculated with *E. coli* O157:H7 at level of log 3 cfu/mL and ripened in embedded type) and SC (cheese milk was inoculated with *E. coli* O157:H7 at level of log 3 cfu/mL and ripened in brined type) groups and at log 5 cfu/mL in GD (cheese milk was inoculated with *E. coli* O157:H7 at level of log 5 cfu/mL and ripened in embedded type) and SD (cheese milk was inoculated with *E. coli* O157:H7 at level of log 5 cfu/mL and ripened in brined type) groups.
The cheese milk was subjected to heat treatment up to approximately 35°C and contaminated with some certain amount of *E. coli* O157:H7 depending on the cheese type. Rennet added milk was allowed to ferment at 30°C for 90 min. After allowing enough time for coagulation, the cheese curd was cut into pieces of 1 x 1 x 1 cm by using special knives. Following this process, the herbs were added at the rate of 2 % of the milk weight and mixed with the coagulum appropriately. Subsequently, the coagulum was transferred into the containers with a cheese cloth lined on the top and drained. Then, the cheese whey was drained off by applying pressure on curd for 2 h. After applying pressure, the curd was cut into blocks with a knife each measuring 7 x 7 x 3 cm. The blocks were coated with rock salt at the rate of 3 % of the weight, covered with a cloth and left to settle for 24 h in ambient temperature. After this stage, each cheese groups were divided into two parts for ripening; half intended for the embedding and the rest for the brine. In the group that would be embedded in soil, the cheese blocks were placed into 1 liter of glass jars firmly and the air gaps were filled with small pieces of cheeses. The jars were closed with the lids on which there were pre-opened holes and embedded into the soil in an inverted position and left for ripening at 9-18°C for 90 days. In the brined group, the cheese blocks were placed into plastic cans with the capacity of 5 liters, then fresh brine of 16 % was added and the lids were closed tightly. Brine water was replaced with fresh brine approximately after 12 hours and the cheese was allowed to ripen at 4°C for 90 days.

Preparation of Samples for Analysis

Each of the cheese samples was weighed out 10 g, transferred into stomacher bags and homogenized for 2 min. by adding 90 mL peptone-saline solution in a stomacher (Laboratory Blender Stomacher 400, Seward, London, UK). Further decimal dilutions were prepared from 1/10 diluted homogenates by means of this method. The duplicate samples were inoculated to the related medium and mean values were counted (Harrigan, 1998).

Microbiological Analysis

Total mesophilic bacteria were counted on Plate Count Agar (Oxoid, Hampshire, UK) incubated at 32°C for 48 h. Lactic acid bacteria were grown on M17 Agar (Oxoid, Hampshire, UK) at 35°C for 48 h. Enterobacteriaceae counts were determined on Violet Red Bile Glucose Agar (Oxoid, Hampshire, UK) at 30°C for 3 days. Micrococci/staphylococci were counted on Baird-Parker Agar (Oxoid, Hampshire, UK) at 37°C for 24-48 h (Pichhardt, 1993). The numbers of *E. coli* O157:H7 were estimated by using the Most Probable Number (MPN) method (ISO, 1984; Farmer and Davis, 1985; Ansay and Kapsar, 1997).

Physico-chemical Analysis

The pH levels of all cheese samples were measured by pH meter (Model 890; Nel Instruments Inc., Ankara, Turkey) (Metin and Öztürk, 2002), and the water activities (a_w) were measured using a water activity meter (Lufft aw, Wert-Messer, Germany) (Fontana, 2002). The salt amount and titratable acidity values were also determined by Kurt et al. (1993).

Results and Discussion

The number of *E. coli* O157:H7 was determined as 3.81 log MPN/g in GC and SC groups, and 5.15 log MPN/g in GD and SD groups at the beginning of ripening period. But, it was found to maintain a regular decline in both embedded and brined

herby cheeses during the ripening period. In embedded herby cheese, the highest decline in *E. coli* O157:H7 level was observed on the 7th day of ripening. While the amount of *E. coli* O157:H7 was found as 1.48 (GC) and 1.40 log MPN/g (GD) on day 60, it was dropped below the detectable limit on day 90. However, the decline progressed slower in brined herby cheese. The number of *E. coli* O157:H7 was detected as 2.40 (SC) and 4.54 (SD) log MPN/g on day 60, and 2.30 (SC) and 4.48 log MPN/g (SD) on day 90 of ripening in brined herby cheese. All the microbiological and chemical changes throughout 90 day-storage are given in Table 1. Furthermore, survival and growth of *E. coli* O157:H7 during ripening of the cheese is shown Figure 1.

Herby cheese has been widely produced and consumed in eastern and southeastern regions of Turkey for a long time. It is famous cheese in these regions, and its popularity is continuously increasing in the rest of Turkey. The major part of herby cheese production is made traditionally at family level or in small cheese plants by using raw milk (Coşkun and Tunçtürk, 1998). In many countries, unpasteurized milk was used for cheese making for centuries and is still being used especially in small farms producing unique type of cheese (Marek et al., 2004).

Pathogenic bacteria such as *Salmonella*, *L. monocytogenes* and enteropathogenic *E. coli* have been categorized as high risk organisms to the cheese industry (Zottola and Smith, 1991). Moreover, outbreaks of foodborne illnesses due to different cheeses from several countries have also been reported (IFST, 1998).

Previous studies indicated that *E. coli* O157:H7 may survive during manufacture and ripening of cheese. Generally, the number of the microorganism drop continuously during storage (Reitsma and Henning, 1996; Ramsaran et al., 1998; Küplülü et al., 1999; Maher et al., 2001; Özer et al., 2004).

In the present study, *E. coli* O157:H7 survived up to 60 days in embedded herby cheese, however, it was found to be eliminated completely at both inoculation levels at the end of the ripening period. In the brined herby cheeses, the counts of *E. coli* O157:H7 decreased continuously during ripening period, and this organism survived up to 90 days at both inoculation levels. The population of *E. coli* O157:H7 were determined lower than the 1st day at the 60th day in GC and GD groups of embedded herby cheeses with 2.33 and 3.75 unit (log MPN/g), respectively. The decrease was slower in brined herby cheese. When compared to 1st day, at the 60th day of the ripening period, *E. coli* O157:H7 declined 1.41 and 0.61 unit (log MPN/g) in SC and SD groups of brined herby cheese, respectively. At day 90, the counts of *E. coli* O157:H7 decreased 1.51 and 0.67 unit (log MPN/g) in SC and SD groups in brined herby cheese, respectively.

Many factors, such as competitive flora, starter culture, heat, pH value, salt, a_w value, inoculation level, cheese production method and storage conditions effect the growth of *E. coli* O157:H7 in cheese (Reitsma and Henning, 1996; Ramsaran et al., 1998; Glass et al., 1998; Küplülü et al., 1999; Maher et al., 2001; Spano et al., 2003; Özer et al., 2004).

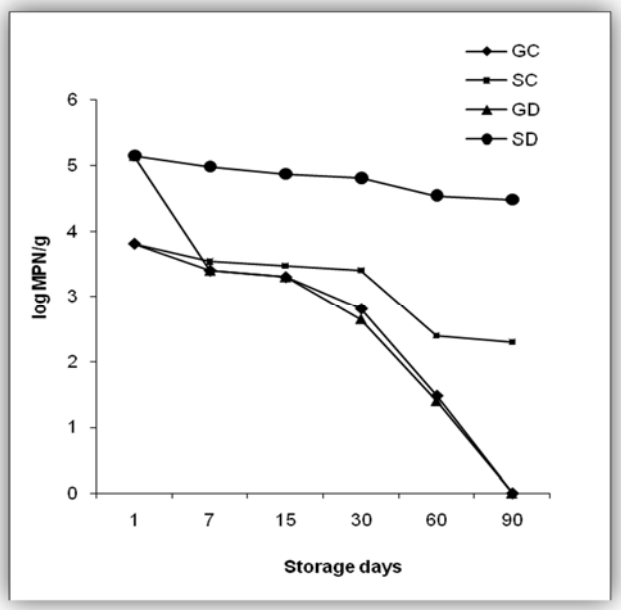

Figure 1. Survival and growth of *E. coli* O157:H7 during ripening of herby cheese

Table 1. The results of microbiological and chemical analysis in herby cheese

Parameters	Cheese type	Storage days					
		1	7	15	30	60	90
E. coli O157:H7 (log MPN/g)	GC	3.81	3.40	3.30	2.81	1.48	ND
	SC	3.81	3.54	3.48	3.40	2.40	2.30
	GD	5.15	3.40	3.30	2.65	1.40	ND
	SD	5.15	4.98	4.87	4.81	4.54	4.48
Total mesophilic bacteria (log cfu/g)	GC	9.38	9.25	7.90	8.58	8.41	8.41
	SC	9.45	9.34	7.90	9.04	8.84	8.78
	GD	9.78	9.08	8.78	8.50	8.56	8.50
	SD	9.45	9.00	9.20	9.11	9.04	8.90
Lactic acid bacteria (log cfu/g)	GC	9.45	9.15	8.45	8.70	8.34	8.60
	SC	9.60	9.00	8.60	8.81	8.15	9.08
	GD	9.64	9.00	9.00	8.48	8.45	8.62
	SD	9.34	9.20	8.78	9.15	8.88	8.87
Micrococci/ Staphylococci (log cfu/g)	GC	5.90	6.32	6.99	7.18	7.50	7.87
	SC	4.30	5.30	5.60	6.93	7.41	7.98
	GD	6.56	6.00	6.15	7.20	7.75	7.86
	SD	5.08	5.28	6.00	7.34	7.71	7.95
Enterobacteriaceae (log cfu/g)	GC	6.78	6.90	5.60	3.70	2.30	ND
	SC	7.56	7.08	5.30	6.50	4.78	6.62
	GD	7.41	6.60	6.20	3.78	2.30	ND
	SD	7.08	7.20	7.08	7.00	4.15	6.66
Titratable acidity (L.A. %)	GC	0.54	0.87	0.89	0.92	1.74	1.80
	SC	0.54	0.68	0.74	0.98	1.40	1.60
	GD	0.51	0.70	0.86	0.96	1.61	1.75
	SD	0.68	0.80	0.84	1.14	1.60	1.70
pH	GC	4.57	4.90	4.92	4.94	4.96	5.51
	SC	4.57	4.40	4.21	4.20	4.16	4.51
	GD	4.91	4.96	4.87	4.85	4.90	5.73
	SD	4.91	4.70	4.60	4.51	4.43	4.62
Salt (%)	GC	2.60	2.92	3.20	3.80	4.09	4.68
	SC	7.25	7.80	8.77	9.00	9.21	9.36
	GD	3.50	3.86	4.12	4.30	4.45	4.56
	SD	7.61	8.00	8.19	8.49	8.75	9.59
a_w	GC	0.97	0.96	0.95	0.94	0.92	0.90
	SC	0.96	0.95	0.94	0.93	0.92	0.91
	GD	0.97	0.96	0.94	0.93	0.91	0.90
	SD	0.96	0.95	0.94	0.92	0.91	0.90

GC: Cheese milk was inoculated with *E. coli* O157:H7 at level of log 3 cfu/mL and ripened in embedded type
SC: Cheese milk was inoculated with *E. coli* O157:H7 at level of log 3 cfu/mL and ripened in brined type
GD: Cheese milk was inoculated with *E. coli* O157:H7 at level of log 5 cfu/mL and ripened in embedded type
SD: Cheese milk was inoculated with *E. coli* O157:H7 at level of log 5 cfu/mL and ripened in brined type
ND: Not detected, L.A.: Lactic acidity, a_w: Water activity

In this study, total mesophilic bacteria, lactic acid bacteria and micrococcus/staphylococcus counts usually had high levels and they shaped the dominant flora (Table 1). The counts of Enterobacteriaceae showed an irregular progression ripening period. While the count decreased to zero in embedded herby cheese after 90 days, it survived at the end of storage in brined herby cheese. Yetişmeyen (1997) reported that coliforms had been completely inhibited in herby cheese for 60 days. Similarly, Coşkun (1998) found that the counts of coliform continuously declined during ripening in herby cheese samples.

Generally, the low pH is mainly caused by the lactic acid produced via lactic acid bacteria and other acids. But, pH values in this study were raised at

the end of storage. This could be due to degradation of neutral form of coagulation contents or the increase of contents that have alkaline features. Likewise, Schlesser et al. (1992) stated that various metabolic degradation products had an effect on pH value. It was reported that mostly the lactic acid produced by lactic acid bacteria, hydrogen peroxide and antibiotic like substances had significant inhibitory effects on pathogenic bacteria (Fernandes and Shahani, 1989).

Another significant factor in growth and development of pathogenic bacteria is salt level. It has a bactericidal and bacteriostatic effect on these bacteria (IDF, 1980). In this study, the salt amount showed a regular increase during the ripening period, it reached up to 4.56-4.68 % level in embedded herby cheese, and 9.36-9.59 % in brined herby cheese at the end of ripening. Also, a_w level had a regular decline during the ripening period which was as low as 0.90-0.91 at the end of ripening.

Inactivation of *E. coli* O157:H7 is mainly due to salt, acidity and storage temperature and time in cheese making. Also, there is a synergistic effect among them (Guraya et al., 1998). Survival of *E. coli* O157:H7 has shown significant change in different cheese varieties (Reitsma and Henning, 1996; Hudson et al., 1997; Ramsaran et al., 1998; Saad et al., 2001; Maher et al., 2001).

In this study, *E. coli* O157:H7 counts demonstrated a regular decline in both embedded and brined cheese, however its followed by different patterns of progression. The antibacterial effects of the herbs used in cheese manufacturing had been reported by some researchers (Coşkun, 1998; Ağaoğlu et al., 2005) These differences in the progression of these bacteria in two cheese types might be caused from different storage conditions. Conner and Kortrola (1995) reported that the *E. coli* O157:H7 stored at low temperature survived longer than those of the stored at high temperature. Besides, increased salt amount might have inhibited the acidity in brined cheese. *E. coli* O157:H7 trend in brined herby cheese is similar to those reported by many researchers (Maher et al., 2001; Saad et al., 2001; Özer et al., 2004).

Conclusions

According to the results obtained in this study, *E. coli* O157:H7 survived at least 60 days in embedded herby cheese, but at the end of ripening period it was eliminated completely in both inoculation levels. However, in brined herby cheese, it survived until the end of ripening period. There is a potential health hazard for the public that consume cheese as fresh or before the ripening period is completed. Therefore, more attention should be paid to the microbiological quality of raw milk, cowhouse hygiene, udder health protection, education of the staff for general health practice and unbroken cold chain. Additionally, taking safety measures such as using pasteurized milk for cheesemaking, preventing the contamination after pasteurization and applying HACCP principles in cheese factories would be beneficial for consumer health.

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