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THE EFFECT OF SALT CONCENTRATION AND pH ON THE SURVIVAL AND GROWTH OF *E. COLI* O157:H7 IN WHITE CHEESE AND TRYPTICASE SOY BROTH

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The survival and growth of E. coli O157:H7 in white cheese during storage at 7 °C and the effect of NaCl concentration (0.5 to 3%) and pH (3.5 to 6.5) in trypticase soy broth on the survival and growth of E. coli O157:H7 at 7°C and 20 °C was investigated.

The number of E. coli O157:H7 was determined by surface plating decimal dilutions of the artificially contaminated white cheese after 0, 5, 10, 15 and 20 days storage at 7°C. Trypticase soy broth of pH 3.5, 5 and 6.5 or with a salt concentration of 0.5%, 2% and 3% was inoculated with 10^5 E. coli O157:H7 and examined after 0, 1 and 5 days storage at 7°C and 20°C on Fluorocult E. coli O157:H7 agar plates following incubation in air at 37°C.

E. coli O157:H7 survived in the white cheese at 7°C and was reduced by 1 log after 10 days and then remained at the same value up to day 20. In the same time the pH fell from 4.02 to 3.35 after 10 days and then slightly increased. The concentration of salt increased from 0.52% to 0.73%. At a pH level of 3.5 in the trypticase soy broth at 7°C E. coli O157:H7 survived but did not grow and the number of pathogens was reduced by 1 log after 5 days. At pH 5 and 6.5 the number of E. coli O157:H7 in the trypticase soy broth increased at both 7°C and 20°C. The number of E. coli O157:H7 in the broth at pH 5 kept at 7°C, increased by 1.7 log, after one day and 3.5 log after five days. At pH 6.5 in the trypticase soy broth kept at 20°C the populations of E. coli O157:H7, increased by 3.5 log, after one day and 4.5 after five days. The organism grew (up to 2 log increase) in the trypticase soy broth containing 0.5%, 2% and 3% of salt at 20°C but did not grow at 7°C and decreased by 0.5 log, at 0.5% salt, 1 log at 2% salt and 2 log at 3% salt, after five days.

This study indicates that E. coli O157:H7 may persist in white cheese. If this pathogen should contaminate low salt products with a pH greater than 4.0, the survival could be sufficient to cause illness. The risk of an outbreak is highly dependent on pH, salt content and storage temperature of the white cheese, but since inactivation is slow and marginal for this type of cheese, the initial level of contamination is critical in determining the safety of white cheese.

Key words: E. coli O157:H7, white cheese, pH, salt concentration, survival, growth

INTRODUCTION

The bacterium, *Escherichia coli*, is normally considered non-pathogenic and can be isolated routinely from the intestinal tracts of warm-blooded and cold-blooded animals, including humans. However, some *E. coli* strains are pathogenic, with specific strains such as O157:H7 causing severe diarrheal syndromes (Padhye *et al*., 1992). Since 1982, when *Escherichia coli* O157:H7 was first identified as a cause of food borne illness (Wells *et al*., 1983) this pathogen has been involved in other outbreaks in which raw milk was the suspected or confirmed vehicle of infection (Martin *et al*., 1986). In an outbreak associated with the consumption of pasteurized milk in Scotland in 1994, more than 100 people were affected (Simmons, 1997).

According to the International Commission on Microbiological Specifications for Foods (1996) growth limiting parameters for pathogenic *E. coli* are minimum temperature 7-8°C, maximum temperature 44-46°C, pH 4.4, water activity 0.95 and sodium chloride 8.5%. Growth has been demonstrated in milk at 6.5 °C (Kauppi *et al*., 1996) and at 7°C (Radenkov *et al*., 1999). *E. coli* O157 is reported to survive at pH levels below 4.4 (Semanchek and Golden, 1996). The pathogen survived largely unchanged in numbers in raw milk held at 7°C for 144 hours and increased by 2.0 $\log_{10}$ at 15°C after 144 hours (Huvelink *et al*., 1998).

As part of an effort to characterize the growth kinetics of *E. coli* O157:H7 Buchanan *et al*. (1992) and Satherland *et al*. (1995) examined the effects of incubation temperature, initial pH, sodium chloride, and sodium nitrite concentration. Both groups found that as incubation temperature decreased, as sodium concentration increased, and as pH decreased, the growth of *E. coli* O157:H7 decreased.

In view of this organism's high acid tolerance (including the ability to survive the acidic environment of the stomach), its resistance to fermentation by-products, its survival during storage at 4 °C, and high salt tolerance, it may be expected to survive in white cheese. The survival and growth of *E. coli* O157:H7 in cheese is an important food safety issue.

Therefore, the purpose of this investigation was to determine the survival and growth of *E. coli* O157:H7 in white cheese and the effect of NaCl concentration (0.5%, 2% and 3%) and pH (3.5, 5 and 6.5) in trypticase soy broth on the survival and growth of *E. coli* O157:H7 at 7°C and 20°C.

MATERIALS AND METHODS

Preparation of E. coli inoculum. *E. coli* O157:H7 was grown at 37°C, in brain heart infusion broth. The test culture was subjected to a minimum of three successive 20-h transfers, before the experiment.

Inoculation and analysis of white cheese. White cheese (moisture 58.71%; fat in dry matter 48.44%; pH 3.98; sodium chloride 0.52 %) was inoculated with a dilution (made in sterile 0.9% saline) to yield a starting count of ca. 10^4 *E. coli* O157:H7/g. The inoculated white cheese was stored at 7°C, for a period of 20 days.

Before inoculating white cheese with *E. coli* O157:H7, the background microbial population was determined. Samples of artificially contaminated white cheese were taken at 0, 5, 10, 15 and 20 days for pH measurement, determination of the salt concentration and microbial analysis.

Physicochemical analysis. The amount of sodium chloride was determined by IDF/ISO/AOAC (88A:1988) methods and hydrogen ion concentration using a pH meter (Extech instruments, microcomputer pH vision 246071) at each sampling time for the enumeration of *E. coli O157:H7*.

Survival E. coli O157:H in trypticase soy broth. The trypticase soy broth was adjusted to pH values of 3.5, 5 and 6.5 using DL- lactic acid. In the second trial trypticase soy broth was supplemented with 0.5%, 2% and 3 % NaCl. In each trial 10 ml of trypticase soy broth was inoculated with a suspension of *E. coli* O157:H7 to provide a resulting population of ca 10^5/ml. One set was stored at 7°C and the other at 20 °C, for a period of 5 days. The experiment was repeated three times.

Enumeration E. coli O157:H7. Duplicate samples of white cheese (20 g) were dispersed in 180 ml of 2% sodium citrate solution. Decimal dilutions of white cheese or trypticase soy broth were plated on to Fluorocult *E. coli O157:H7* agar plates (Merck-Germany). The plates were incubated in air at 37°C, for 24 h and typical greenish sorbitol negative colonies were counted and confirmed by biochemical tests and the latex agglutination test for *E. coli* O157 (Test Kit; Oxoid).

Enumeration of total viable bacteria. The total bacterial counts in white cheese were determined in decimal dilutions of white cheese in sterile 0.9% saline. They were plated on to total bacterial count agar plates (Torlak-Yugoslavia) and incubated at 30-32°C for 72^h.

RESULTS

The results concerning the survival of *E. coli* O157:H7 in white cheese stored at 7 °C for 20 days are shown in Table 1.

Table 1. Change in *E. coli O157:H7* numbers, total bacterial counts, pH and amounts of sodium chloride in white cheese storaed at 7°C

Time (days)	*E. coli O157:H7* (log CFU/g)*	*Total bacterial counts* (log CFU/g)*	*pH**	*Salt concentration**
0	*4.18*	*7.87*	*3.98*	*0.52*
5	*4.11*	*7.85*	*3.91*	*0.66*
10	*3.54*	*8.06*	*3.76*	*0.69*
15	*3.17*	*7.95*	*3.53*	*0.73*
20	*2.16*	*7.85*	*3.92*	*0.79*

*Mean values of three determinations

The white cheese with 0.52% NaCl and pH 3.98 was produced from raw milk. During storage at 7°C, the salt concentration increased from 0.52% to 0.79%.

The pH stayed nearly the same in the first five days, then decreased up to day 15 and then increased. The total bacterial counts remained nearly constant, during storage of white cheese.

For acidic products such as white cheese, in which lactic acid is produced by the metabolic activity of bacteria fom the starter cultures, pH contributes to controlling *Escherichia coli* O157:H7 growth. However, *Escherichia coli* O157:H7 can survive in white cheese kept for 20 days at refrigeration temperatures (7°C). Thus, the count remained the same in the first five days, and then decreased by 0.5 log cycle after ten days and 2 log after 20 days.

Therefore we investigated the behavior of *Escherichia coli* O157:H7 at 7°C and 20°C in the trypticase soy broths at pH 3.5, 5 and 6.5 and sodium chloride concentration of 0.5 %, 2% and 3%, which range could be expected in this kind of cheese. The results of these investigations are shown in Table 2 and Table 3.

Table 2. The effect of pH and sodium chloride concentration on the survival and growth of *Escherichia coli* O157:H7 in trypticase soy broth stored at 7 °C

Time (days)	*Escherichia coli* O157:H7 (log CFU/g)*					
	pH			Sodium chloride (%)		
	3.5	5	6.5	0.5	2	3
0	5.63	5.58	5.61	5.59	5.91	5.62
1	5.60	7.23	8.11	6.66	6.04	5.34
5	5.50	8.23	8.27	6.02	4.48	3.54

*Mean values of three determinations

Table 3. The effect of pH and sodium chloride concentration on the survival and growth of *Escherichia coli* O157:H7 in trypticase soy broth stored at 20 °C

Time (days)	*Escherichia coli* O157:H7 (log CFU/g)*					
	pH			Sodium chloride (%)		
	3.5	5	6.5	0.5	2	3
0	5.58	5.45	5.61	5.39	5.30	5.60
1	5.29	7.11	8.11	6.68	5.70	5.00
5	3.42	8.27	9.29	7.12	6.12	4.11

*Mean values of three determinations

Escherichia coli O157:H7 survived but its number slowly decreased in the trypticase soy broth at pH 3.5 kept at the temperature of 7 °C for five days (Table 2). Growth occurred in the trypticase soy broth kept at pH 5 with a 1.65 - 2.65 log cycle increase in population, at 1 to 5 days. In the trypticase soy broth adjusted to pH 6.5 the number of *E. coli* O157:H7 increased by 2.50 log cycles after one day and then remained nearly the same up to five days. Slow growth was observed in

the trypticase soy broth with 0.5% sodium chloride with a 1 log cycle increase in population after one day and then a decrease. In the trypticase soy broth with 2% sodium chloride the number of *Escherichia coli* O157:H7 was nearly the same after one day and then decreased by 1.43 log cycle after five days. *Escherichia coli* O157:H7 survived, but did not grow, in the trypticase soy broth with 3% of sodium chloride. The count remained nearly the same after one day and then exhibited a 2 log cycle reduction in population after five days.

In the trypticase soy broth at pH 3.5 and at the temperature of 20 ^{o}C, *Escherichia coli* O157:H7 survived for five days with a 2.16 log cycle reduction in population. Growth occurred in the trypticase soy broth at pH 5 with a 1.66 - 2.82 log cycle increase in population, at 1-5 days. In the trypticase soy broth at pH 6.5 the number of *E. coli* O157:H7 was increased by 2.50 log cycles after the first day and 3.68 log cycles after five days. Also growth, was demonstrated in the trypticase soy broth with 0.5% sodium chloride with an increase in population of 1.73 log cycles, after five days. Slow growth was demonstrated in the triptycase soy broth containing 2% of sodium chloride with an increase of 0.82 log cycles in population, after five days. Growth of *Escherichia coli* O157:H7 did not occur in the trypticase soy broth with 3% of sodium chloride, but the pathogen survived for five days with a reduction of 1.49 log cycle in its population (Table 3).

DISCUSSION

The results of this study indicated that *Escherichia coli* O157:H7 could survive sufficiently well in white cheese to be of concern. Reitsma and Henning (1996) reported the survival of *Escherichia coli* O157:H7 during the manufacture and curing of cheddar cheese. Govaris *et al*. (2002) found that *Escherichia coli O157:H7* survived manufacture with thermophilic and mesophilic starters of Feta and Telemes cheeses, but was not detectable in Feta cheese after 44 to 36 days ripening and in Telemes cheese after 40 to 30 days for the trials, respectively (Govaris *et al*., 2002). The results obtained in our study also confirm the data from the model system of Guraya *et al*. (1998) that products at or below pH 4.1 could still be vehicles for *Escherichia coli* O157:H7, if they were consumed soon after being manufactured or if they were contaminated with high levels of the pathogen. This indicated that pH and sodium chloride concentration are probably the primary mechanisms by which this pathogen is inhibited in white cheese.

Thus, the pathogen survived, but did not grow in our trypticase soy broth at pH 3.5 either 7^{o}C or 20 ^{o}C. The reduction in population of *Escherichia coli* O157:H7 was greater at 20 ^{o}C. However, growth was observed in trypticase soy broth at pH 5 and 6.5 at both temperatures, especially at 20 ^{o}C. This confirmed the results of a previous study, that *Escherichia coli* O157:H7 can survive at pH 3.4 when it is acid adapted (Lin *et al*, 1995) and at 6.5% of sodium chloride (Glass *et al*. 1992) and at refrigeration temperatures (Conner, 1992). Similar results have been observed in other survival and growth studies. Abdul-Raouf *et al*. (1993) nouted growth of *Escherichia coli* O157:H7 at 20 ^{o}C, but not at 5 ^{o}C. Conner and Kotrola (1995) reported the absence of growth of *Escherichia coli* O157:H7, but the pathogen survived, in acid conditions (pH 4.0) at 5 ^{o}C. The fate of *Escherichia*

coli O157:H7 at low pH has already been studied. Glass *et al*. (1992) found that in trypticase soy broth adjusted with lactic acid to pH 4.5, the cells were inactivated to undetectable levels, and no viable cells were detected at pH 4.0, and 3.5 after 7 days. Clavero and Beuchat (1996) reported the survival of this pathogen at pH 4.8 and 5 and at the temperatures of 20°C and 30°C. It has been reported that *Escherichia coli* O157:H7 can grow at a pH as low as 4.4, although its exact tolerance is influenced by the specific acid present (Semanchek *et al*., 1996). Increased acid tolerance offers such an organism ecological advantages by allowing the pathogen to survive in the gastric system and then to establish infection in the colon and other organs (Arnold *et al*., 1995).

The ability of *Escherichia coli* O157:H7 to survive such adverse conditions, and recognition of its low infectious dose, means that its presence in white cheese is particularly undesirable. This study indicated that *Escherichia coli* O157:H7 may persist in the white cheese until the time of consumption. The risk of an outbreak is highly dependent on pH, salt content, and storage temperature of the product, but since inactivation is slow and marginal for white cheese, the initial level of contamination is the critical factor in determining its safety.

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UTICAJ KONCENTRACIJE SOLI I PH NA PREŽIVLJAVANJE I UMNOŽAVANJE *ESCHERICHIA COLI* O157:H7 U BELOM SIRU I TRIPTOZA SOJA BUJONU

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SADRŽAJ

Ispitivano je preživljavanje i umnožavanje *E. coli* O157:H7 u belom siru čuvanom pri 7 °C i uticaj koncentracije NaCl (0.5% 2% i 3%) i pH (3.5, 5 i 6.5) u triptoza soja bujonu na preživljavanje i umnožavanje *E. coli* O157:H7 pri 7 °C i 20 °C.

Broj *E. coli* O157:H7 je određivan zasejavanjem decimalnih razblaženja eksperimentalno kontaminiranog sira, čuvanog 0, 5, 10, 15 i 20 dana na 7 °C i triptoza soja bujona pH 3.5, 5 i 6.5 ili triptoza soja bujona sa 0.5%, 2% i 3% NaCl, inokulisanog sa 10^5 *E. coli* O157:H7 posle 0, 1 i 5 dana čuvanja na 7 °C i 20 °C, na Fluorocult *E. coli* O157:H7 agar. Zasejane podloge su inkubirane pri 37 °C.

E. coli O157:H7 je preživljavala u belom siru pri 7 °C uz redukciju broja za 1 log posle deset dana, koji je zatim ostao nepromenjen do 20 dana. U isto vreme pH belog sira se snižavala sa 4.02 na 3.35 posle deset dana, a potom se polako

povećavala. Koncentarcija natrijum hlorida se povećavala sa 0.52% na 0.73%. *E. coli* O157:H7 je preživljavala, ali se nije umnožavala u triptoza soja bujonu pri pH vrednosti 3.5 i temperaturi od 7 °C, uz redukciju broja za 1 log vrednost posle pet dana. U triptoza soja bujonu, pri pH 5 i 6.5, broj *E. coli* O157:H7 se povećavao pri obe temperature 7 °C i 20 °C. U triptoza soja bujonu pH 5 broj *E. coli* O157:H7 se pri 7 °C povećavao za 1.7 log, posle jednog dana i 3.5 log posle pet dana. Pri pH vrednosti od 6.5 u triptoza soja bujonu i temperaturi od 20 °C populacija *E. coli* O157:H7 se povećavala za 3.5 log posle jednog dana i 4.5 log posle pet dana. Patogen je rastao (do 2 log povećanje) pri 20 °C u triptoza soja bujonu sa sve tri koncentracije NaCl (0.5%, 2, 3%). *E. coli* O157:H7 se nije umnožavala pri istim koncentracijama NaCl i temperaturi od 7 °C već se posle pet dana broj patogena redukovao za 0.5 log pri 0.5% natrijum hlorida, 1 log pri 2% natrijum hlorida i 2 log pri 3% natrijum hlorida.

Ova studija pokazuje da *E. coli* O157:H7 može da perzistira u belom siru. Ukoliko ovaj patogen kontaminira proizvode sa malim sadržajem soli i pH većim od 4.0, mikroorganizam preživljava u dovoljnom broju da izazove bolest. Rizik od pojave alimentarnog oboljenja u mnogome zavisi od pH, koncentracije soli i temperature čuvanja belog sira, ali pošto je inaktivacija patogena spora i neznatna u toj vrsti sira, početni nivo kontaminacije je kritičan za utvrđivanje higijenske ispravnosti belog sira.