Survival of \textit{E. coli} O157:H7 in Yogurt Incubated until Two Different pH Values and Stored at 4°C [1]

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\textbf{INTRODUCTION}

Yoghurt is a dairy product produced by bacterial fermentation of milk. Fermentation of the milk sugar (lactose) produces lactic acid, which acts on milk protein to give yoghurt its characteristic textural and sensorial properties. It is believed that yoghurt originated in the Middle East, and the evolution of this fermented product through the ages can be attributed to the culinary skills of the nomadic people living in that part of the world \footnote{1}. The word is derived from Turkish “\textit{yoğurt}” \footnote{2}. Yoghurt is one of the most important dairy products and are consumed approximately 2.293.431 tons/year in Turkey \footnote{4}.

The serotype O157:H7 among \textit{E. coli} strains is highly virulent. This serotype not only associated with a number of highly publicized food-borne outbreaks, but also it is able to survive acidic conditions \footnote{5}. \textit{Escherichia coli} O157:H7, a Gram-negative, facultative anaerobe, is a food-borne pathogen well-known in the dairy and food industries \footnote{6}. It was first recognized as a pathogen in 1982 \footnote{6,8} and is considered to be an important causative agent of diarrhoea, hemorrhagic colitis and hemolytic uremic syndrome (HUS) \footnote{8}. It has been identified as the causative agent of severe enteric illness which can strike suddenly, affect all
ages, has a low infectious dose and can cause death \(^{10}\). Foods of animal origin were reported to be the primary sources of \(\text{E. coli} \ O157:H7\) infection \(^{11}\).

Subsequent outbreaks, which predominantly involve the \(\text{E. coli} \ O157:H7\) serotype, have been reported in foods including minced beef, cheese, sprouts, salami, and apple cider \(^{1}\). Several authors have demonstrated that \(\text{E. coli} \ O157:H7\) can survive in dairy products over several days and weeks: yoghurt and Colby, Romano and Feta cheeses, Cheddar cheese, sour cream, buttermilk, and cheese, goat milk lactic cheeses and Cheddar cheese whey \(^{12-17}\). It has been observed that \(\text{E. coli} \ O157:H7\) inoculated into yoghurt may remain viable for a few hours at 30-40°C, and up to 1-8 days while refrigerated \(^{18}\). The survival of this microorganism for up to several weeks illustrates the potential health risks associated with post-processing contamination of even low levels of \(\text{E. coli} \ O157:H7\) in several dairy products \(^{13}\). It is reported that there was only 1 outbreak caused by \(\text{E. coli} \ O157:H7\) involving yoghurt \(^{19}\) but possibility of contamination does exist \(^{18}\).

According to different research \(\text{pH}\) values of yoghurt produced in Turkey varies between approximately 3.5-4.6 \(^{20-23}\). Strains of \(\text{E. coli} \ O157:H7\) have been found to be relatively acid-tolerant and the infectious dose is less than 50 cells \(^{24}\). Hence in this study, two different \(\text{pH}\) values and two different contamination levels were selected. The aim of this study was to evaluate the behavior of \(\text{E. coli} \ O157:H7\) in yoghurts produced at two different initial \(\text{pH}\) values and stored at 4°C during 14 days storage period.

**MATERIAL and METHODS**

**Yoghurt Production**

Cow’s milk was used to prepare yoghurt samples. Milk was standardized to approximately 3.8% fat and 12% fat-free dry matter content (according to the Turkish Food Codex Fermented Milks Regulation (2009/25) for full-fat yoghurt) \(^{25}\). Fat-free dry matter of the milk was standardized with fat-free milk powder (Pınar A.S., İzmir, Turkey) and its fat with cream. Standardized milk was heated to approximately 90°C for 5 min and then cooled to 45°C in a water bath.

Commercial Direct Set Lyophilized starter (DELVO-YOG CY-223-DSL, Delft, The Netherland) including \textit{Streptococcus thermophilus} and \textit{Lactobacillus delbrueckii} ssp. \textit{bulgaricus} was used as the starter culture and added to standardized milk at levels recommended by the manufacturer.

Inoculated milk was divided into three groups. The first group was not contaminated with \textit{Escherichia coli} O157:H7 and used as the control group. The other two groups were separately inoculated with dilutions of \textit{Escherichia coli} O157:H7 to achieve an initial population of \(ca. 10^3 \text{ cfu}/\text{mL}\) (low inoculation) and \(ca. 10^5 \text{ cfu}/\text{mL}\) (high inoculation) respectively.

All the samples control and contaminated with different level of \(\text{E. coli} \ O157:H7\) distributed into 100 mL sterile glass jars and incubated at 42°C. Fermentation process was continued until the desired acidity (pH 4.6 and pH 4.0) was reached. Fermentation of half of the samples in the groups was finished at pH 4.6 and the other half was finished at pH 4.0. After fermentation, contaminated and the control group yoghurt samples were stored at 4°C for 14 d. In the samples, viable counts of \(\text{E. coli} \ O157:H7\), pH and titratable acidity were determined on day zero and on the 1\(^{st}\), 2\(^{nd}\), 4\(^{th}\), 6\(^{th}\), 8\(^{th}\) and 14\(^{th}\) days of storage.

**Preparation of \textit{Escherichia coli} O157:H7**

\(\text{E. coli} \ O157:H7\) was kindly provided by Prof. S. A. Aytaç (Hacettepe University, Faculty of Engineering, Food Engineering Department, Ankara, Turkey) \(^{26}\). Culture was transferred into brain-heart infusion (BHI) broth (Oxoid, UK) at 35±2°C for 24 h. Culture grown 24 h was inoculated on cefixime tellurite sorbitol MacConkey agar (CT-SMAC) at 35-37°C for 24 h. White-colorless colonies on CT-SMAC were transferred into modified tryptic soy (MTS) broth (Oxoid, UK) at 37°C for 24 h. Then, serial dilutions were prepared and bacterial counts were determined for using yoghurt samples. \textit{Escherichia coli} O157:H7 involving initial inoculums levels of \(ca. 10^3 \text{ cfu}/\text{mL}\) and \(ca. 10^5 \text{ cfu}/\text{mL}\) was inoculated into yoghurt samples.

**Enumeration of \textit{Escherichia coli} O157:H7**

The glass jars were shaken just before sampling and 1 mL samples were diluted (w/v), in duplicate, in sterile peptone water (Oxoid, UK). 0.1 mL portions from appropriate dilutions were surface-plated onto Sorbitol MacConkey agar (SMAC-Oxoid, UK) including Cefixime Tellurite Selective Supplement (CT-Oxoid, UK). The plates were incubated at 35±2°C for 48 h. After incubation, random selected colonies were confirmed by serology with the \textit{E. coli} O157 latex test (Oxoid, UK).

**Chemical Analysis**

The pH of the yoghurt samples was measured with a HANNA pH meter (HANNA Instruments, Italy). The titratable acidity (TA, as percent lactic acid) was determined according to the method of AOAC \(^{27}\).

**Statistical Analysis**

Data obtained from experiments was analyzed using SPSS software version 8.00 \(^{28}\). Variance analyses and Tukey’s multiple comparison tests were used to determine significance in the determined characteristic between the samples produced at 4.0 and 4.6 initial pH and contaminated with \(10^3\) and \(10^5 \text{ cfu}/\text{mL}\) \(\text{E. coli} \ O157:H7\). Log\(_{10}\) transformations were performed on microbial data.
Principal Components Analysis (PCA) was also performed to show relationships among groups of variables and between objects.

**RESULTS**

Viable counts of *E. coli* O157:H7 showed a slightly increase in milk during fermentation period at 42°C. Counts of *E. coli* O157:H7 in yoghurt samples produced at two different initial pH, contaminated ca. 10^3 or 10^5 cfu/mL pathogen and stored at 4°C were given in Fig. 1 and Fig. 2. During the 14-day storage period, *E. coli* O157:H7 was not found in any uncontaminated yoghurt control samples. In the yoghurt samples produced 4.6 initial pH and contaminated low level *E. coli* O157:H7, counts of *E. coli* O157:H7 decreased from 3.93 log<sub>10</sub> cfu/mL to 2.19 log<sub>10</sub> cfu/mL on day 0 and 6<sup>th</sup> respectively. Viable cells number of *E. coli* O157:H7 in these samples decreased to undetectable level after 8 days. In the same samples contaminated with high level pathogens, the counts of *E. coli* O157:H7 reduced to 2.25 log<sub>10</sub> cfu/mL on day 14<sup>th</sup>.

In the present study, *E. coli* O157:H7 numbers in yoghurt produced at initial pH level 4.0 and inoculated with 10^5 cfu/mL *E. coli* O157:H7 decreased to 2.07 log<sub>10</sub> cfu/mL after 8 days and, after 14 days viable counts of *E. coli* O157:H7 decreased to undetectable level. In the yoghurt at same pH and with 10^3 cfu/mL contamination level, viable counts of *E. coli* O157:H7 decreased to undetectable level after 4 days.

In all samples produced at 4.0 and 4.6 initial pH and contaminated whit 10^3 and 10^5 cfu/mL *E. coli* O157:H7, differences among the survival of *E. coli* O157:H7 were significant (P<0.001). Survival of *E. coli* O157:H7 was affected (P<0.001) by initial pH of yoghurt, contamination level of *E. coli* O157:H7 and storage time.

Variances in pH values of yoghurt samples produced in the present study were shown in Table 1. The pH values showed a regular variance in samples produced at pH 4.0. The pH of each of the two sample groups contaminated with 10^3 and 10^5 cfu/mL *E. coli* O157:H7 and the control (Day 0 should be excluded as this sample did not contain pathogen) decreased from 4.00 to 3.80 during storage period. The same regular variance in pH levels during storage was observed in the samples produced at pH 4.6. The pH values of these samples decreased from 4.6 to 4.30 at the end of the storage period. The pH values of yoghurt...

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**Fig 1.** Survival of *E. coli* O157:H7 in yoghurt inoculated with 10^5 cfu/mL *E. coli* O157:H7 during storage at 4°C

**Şekil 1.** 10^5 cfu/mL *E. coli* O157:H7 ile kontamine edilen ve 4ºC'de depolanan yoğurtlarda *E. coli* O157:H7'nin canlı kalma durumu

**Fig 2.** Survival of *E. coli* O157:H7 in yoghurt inoculated with 10^3 cfu/mL *E. coli* O157:H7 during storage at 4°C

**Şekil 2.** 10^3 cfu/mL *E. coli* O157:H7 ile kontamine edilen ve 4ºC'de depolanan yoğurtlarda *E. coli* O157:H7'nin canlı kalma durumu
samples were affected (P<0.001) by initial pH of yoghurt and storage time.

Acidity values of all samples increased during storage period (Table 2). In the yoghurt produced at pH 4.6 and contaminated with $10^0$ and $10^5$ cfu/mL $E. coli$ O157:H7, acidity values were determined on day 0 and 14 as 0.76-1.03, 0.85-1.14 and 0.89-1.16, respectively. In the samples produced at pH 4.0 and contaminated by $E. coli$ O157:H7 at different levels, ($0, 10^1$ and $10^5$ cfu/mL) on the same day, titratable acidity values were determined as 1.47-1.70, 1.52-1.72 and 1.48-1.80, respectively. The effect of initial pH of yoghurt, contamination level of $E. coli$ O157:H7 and storage time was significant (P<0.001) on the titratable acidity values of yoghurt samples.

Results of the principal component analysis (PCA) showed that principal components (PC) 1 and 2 described

Table 1. Change of pH in yoghurt produced at 4.0 and 4.6 initial pH during storage at 4ºC

<table>
<thead>
<tr>
<th>Storage Period (Day)</th>
<th>4.00 Initial pH</th>
<th>4.60 Initial pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Low Inoculation</td>
<td>High Inoculation</td>
</tr>
<tr>
<td>0</td>
<td>4.00±0.08</td>
<td>4.00±0.14</td>
</tr>
<tr>
<td>1</td>
<td>4.00±0.14</td>
<td>4.00±0.21</td>
</tr>
<tr>
<td>2</td>
<td>3.90±0.07</td>
<td>4.05±0.24</td>
</tr>
<tr>
<td>4</td>
<td>3.90±0.08</td>
<td>3.90±0.01</td>
</tr>
<tr>
<td>6</td>
<td>3.90±0.01</td>
<td>3.90±0.03</td>
</tr>
<tr>
<td>8</td>
<td>3.90±0.03</td>
<td>3.90±0.01</td>
</tr>
<tr>
<td>14</td>
<td>3.80±0.04</td>
<td>3.80±0.03</td>
</tr>
</tbody>
</table>

Table 2. Change of pH in yoghurt produced at 4.0 and 4.6 initial pH during storage at 4ºC

<table>
<thead>
<tr>
<th>Storage Period (Day)</th>
<th>4.00 Initial pH</th>
<th>4.60 Initial pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Low Inoculation</td>
<td>High Inoculation</td>
</tr>
<tr>
<td>0</td>
<td>1.47±0.01</td>
<td>1.52±0.03</td>
</tr>
<tr>
<td>1</td>
<td>1.49±0.04</td>
<td>1.54±0.01</td>
</tr>
<tr>
<td>2</td>
<td>1.52±0.06</td>
<td>1.58±0.03</td>
</tr>
<tr>
<td>4</td>
<td>1.55±0.03</td>
<td>1.59±0.01</td>
</tr>
<tr>
<td>6</td>
<td>1.57±0.01</td>
<td>1.60±0.04</td>
</tr>
<tr>
<td>8</td>
<td>1.58±0.03</td>
<td>1.64±0.03</td>
</tr>
<tr>
<td>14</td>
<td>1.70±0.04</td>
<td>1.72±0.04</td>
</tr>
</tbody>
</table>
had about 80.9% of the total variation of the events: 49.1% PC1 and 31.7% PC2 (Fig. 3). PC1 was heavily loaded on initial pH, pH and titratable acidity of yoghurt samples; whereas PC 2 was loaded on contamination level of E. coli O157:H7, storage time and counts of E. coli O157:H7. The PCA analysis presented a strong positive correlation between initial pH and pH and a strong negative correlation between initial pH and titratable acidity of samples. The PCA results showed that there was a strong positive correlation between contamination levels of E. coli O157:H7 and counts of E. coli O157:H7 and there was a strong negative correlation between storage time and counts of E. coli O157:H7. Results also showed that there was low positive relationship between the pH and the counts of E. coli O157:H7 and there was high negative relationship between the pH and titratable acidity (Fig. 3).

DISCUSSION

Viable counts of E. coli O157:H7 showed a slight increase in milk during fermentation period at 42°C. Similar result reported by Bachrouri et al.\textsuperscript{30}. On contrary, Massa et al.\textsuperscript{18} stated that the viable counts of E. coli O157:H7 which were inoculated into milk and yoghurt did not change during fermentation at 42°C. Chang et al.\textsuperscript{31} stated that E. coli O157:H7 grow in skim milk fermented with either L. delbrueckii spp. bulgaricus or L. casei spp. casei.

Bachrouri et al.\textsuperscript{30} examined the survival of E. coli O157:H7 during milk fermentation at 43°C and storage of home-made yoghurt (initial pH = 4.6) at refrigeration temperatures (2, 4, or 8°C). They reported that E. coli O157:H7 counts decreased undetectable level after 21 d at 2 or 4°C and after 7 d stored at 8°C. Guraya et al.\textsuperscript{32} reported that, in traditional yoghurts inoculated with 10\textsuperscript{5} cfu/g E. coli O157:H7 after production, E. coli O157:H7 survived at pH 4.65 for 35 days, whereas it survived at pH 4.17 for 7 days. Kasimoğlu and Akgün\textsuperscript{33} studied the survival of E. coli O157:H7 in traditional and acidophilus yoghurts which were inoculated at levels of different doses (10\textsuperscript{3}, 10\textsuperscript{4} and 10\textsuperscript{5} cfu/mL) of E. coli O157:H7. They determined that the elimination times of E. coli O157:H7 in traditional yoghurts were 48 h for 10\textsuperscript{3} and 10\textsuperscript{4} cfu/mL contamination levels and 72 h for 10\textsuperscript{5} cfu/mL contamination level. In their study, elimination times of E. coli O157:H7 were determined as 3 h for 10\textsuperscript{3} cfu/mL, and as 48 h for 10\textsuperscript{4} and 10\textsuperscript{5} cfu/mL in acidophilus yoghurt.

Similarly, in our study, it was determined that in yoghurts inoculated with 10\textsuperscript{3} cfu/mL E. coli O157:H7 and an initial pH of 4.6, the number of E. coli O157:H7 viable counts decreased to 2.6 log\textsubscript{10} cfu/mL as a result of storage period and survived even on the 14\textsuperscript{th} day. E. coli O157:H7 in the yoghurt samples inoculated with 10\textsuperscript{3} cfu/mL pathogen and produced 4.6 initial pH, were not found after 8 day.

Dineen et al.\textsuperscript{15} found that the survival time of E. coli O157:H7 either inoculated into traditional yoghurt (pH 4.0) or into acidophilus yoghurt (pH 4.0), at the post-processing stage, was either 14 or 6 days, respectively. Akdemir-Evrendilek\textsuperscript{22} noticed that E. coli O157:H7 in the plain yoghurt, yoghurt drink and fresh-salted yoghurt inoculated with 10\textsuperscript{6}-10\textsuperscript{7}cfu/mL E. coli O157:H7, survived for 45 days at 4 and 22°C. Bachrouri et al.\textsuperscript{34} studied the behavior of E. coli O157:H7 strains during the storage of plain live yoghurt at 4°C. The researchers reported that E. coli O157:H7 counts decreased from 4.4 log cfu/g to 3.6 log cfu/g after 72 h and was not detected after 312 h. In these samples the pH values ranged from 3.94 to 4.11 during the storage.

E. coli O157:H7, with an optimum growth at pH around 7.0, can grow in the pH range between 4.5 and 9.0\textsuperscript{35}. It has been reported that this pathogen can survive in some acidic foods such as sweet pickle (pH 2.8)\textsuperscript{36}, yoghurt (pH 4.5)\textsuperscript{18} and mayonnaise (pH 3.65)\textsuperscript{37}. Yoghurt has always been considered intrinsically safe because of its acidic nature. However the resistance of these pathogens to low pH enables them to survive in high acidic food such as yoghurt until they are consumed\textsuperscript{15,38-41}.

Massa et al.\textsuperscript{18} investigated the survival of E. coli O157:H7 in traditional and bifidus yoghurt at contamination levels of 10\textsuperscript{3} and 10\textsuperscript{4} cfu/mL. In this research, it was determined that pH values of traditionally-produced yoghurt samples contaminated with low level pathogen changed between 6.6 and 4.5, while it changed between 6.6 and 4.4 in the samples contaminated with high level pathogen in the 0th hour and 7th day. Kasımoğlu and Akgün\textsuperscript{33} studied traditional and acidophilus yogurts which were stored at 4°C. They controlled the pH variances of samples, which they contaminated with E. coli O157:H7 at levels of 10\textsuperscript{5}, 10\textsuperscript{6} and 10\textsuperscript{7} cfu/mL and they found that during the refrigerated storage time of traditional yoghurt samples in all treatments, pH dropped from 4.6 to 4.2 and 3.96 at 24, 48 and 72 h, respectively. A similar result was also reported by Gülmez and Güven\textsuperscript{42}. In the present study also, it was determined that pH values showed similar, regular variances at both contamination levels.

In this study it was found that both the initial pH values of the yoghurt samples and contamination level of the pathogen were significant (P<0.001) factors on the survival of E. coli O157:H7. The results of the present study show that E. coli O157:H7 survived in yoghurt during fermentation and storage period even at low pH. Although yoghurt is produced from heat-treated milk, potential contamination after heat treatment might negatively affect the reliability of the product and present a significant health risk to consumers. Consequently, high-acidic foods such as yoghurt should not be considered as safe. Because some pathogen such as E. coli O157:H7 are resistant to acidic condition and can survive for up to several weeks in several dairy products. According to
results obtained from our study it can be said that post-fermentation contamination and contamination level at storage period are important risk factors regarding the safety of the yoghurt.

REFERENCES