Influence of Yoghurt Starter Culture on Viability of Some Pathogenic Microorganisms in Yoghurt

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ABSTRACT
This study was conducted to investigate the survival of *Escherichia coli* and *Listeria monocytogenes* during the storage period of laboratory manufactured yoghurt. Yoghurt was manufactured from laboratory pasteurized milk experimentally inoculated with *E. coli* ATCC 25922 and *L. monocytogenes* at an approximate population level of $6.5 \times 10^5$ and $3 \times 10^5$ CFU/mL milk, respectively. The influence of starter culture on survival of the pathogenic microorganisms during the storage of laboratory manufactured yoghurt at 4°C for 15 days was studied. The obtained results revealed that the inoculated *E. coli* and *L. monocytogenes* couldn’t be detected in yoghurt samples at 9th and 12th day of the storage period when the titratable acidity percentage was 0.9 and 1.36%, respectively. This inhibitory effect may be attributed to the higher acidity of yoghurt.

Key words: *Escherichia coli*, *Listeria monocytogenes*, yoghurt starter culture

INTRODUCTION
Fermentation technology is one of the oldest known methods of food preservation. Fermentation processes promote the development of essential and safe microflora which play a vital role in preventing the outgrowth of spoilage and food borne pathogens (Gibbs, 1987). Lactic Acid Bacteria (LAB) are important in much fermentation and the antagonistic effects of LAB are attributed to some of their biochemical features. They can utilize carbohydrates and produce organic acids as lactic acid or acetic acid. The majority of food borne contaminants, either pathogenic or non-pathogenic is sensitive to these acids and the resulting low pH. They also produce antibacterial substances such as bacteriocines, hydrogen peroxide, diacetyl and CO$_2$ which may also play part in the antagonism of LAB on other microorganisms (Maganusson and Schnurer, 2001).

*Escherichia coli* and *Listeria monocytogenes* considered as the most common food borne pathogens that are present in many foods and are able to survive in fermented milk products. Many *Escherichia coli* strains are harmless and are commonly found in the intestinal tract of warm-blooded organisms. Other strains such as Vero toxin-producing *E. coli* (VTEC) serotype especially serotype O157:H7, cause serious poisoning in humans (APHA, 2004). *Listeria monocytogenes* is ubiquitous in nature due to its inherent ability to survive and grow under a wide range of adverse environmental conditions, such as refrigeration temperatures, high acidity and salinity and reduced water activity (Gandhi and Chikindas, 2007). According to the European Centre for Disease Control and Prevention, listeriosis was the fifth most common zoonotic infection in Europe in 2006 (EFSA, 2007) while it accounts for approximately 28% of the deaths resulting from food-borne illnesses in the United States (Mead *et al.*, 1999).
In food industry, inadequately cleaned food-processing equipment constitutes a potential source for *L. monocytogenes* (Midelet and Carpentier, 2002). Milk and milk products are frequently incriminated (Rocourt, 1996) among dairy products, yoghurt received the least attention due to the fact that its high acidity and milk pasteurization were thought to be effective barriers to the growth of many pathogens including *L. monocytogenes*. It is now well established that the pathogen survives processing and storage of cultured milks including yoghurt and other dairy products fermented with the same starter (Ribeiro and Carminati, 1996; Schaal and Marth, 1988). According to De Buyser et al. (2001), *L. monocytogenes* was responsible for 10 out of 64 outbreaks implicating dairy products among which 32.8% were made from pasteurized milk. Moreover, reported adaptation of the pathogen to acidity (Gahan et al., 1996; Mazzotta, 2001) is warning us for its possible occurrence in low-acid foods.

This study was conducted to determine the influence of yoghurt starter culture on the viability of some pathogenic microorganisms (*Escherichia coli* and *Listeria monocytogenes*) during the storage period of laboratory manufactured yoghurt.

**MATERIALS AND METHODS**

**Used milk:** Normal clean buffalo’s milk was obtained from the Department of Dairy Production, Faculty of Agriculture, Cairo University. The milk was free from any inhibitory substances as approved by Lactic acid activity test (Kosikowski and Mistry, 1997).

**Reference strain of *Escherichia coli***: Reference strain of *E. coli* (ATCC No. 25922) was used. The organism was inoculated in tryptic soya broth with 0.6% yeast extract, incubated at 37°C for 24 h, then tenth fold serial dilution was made, the inoculation level was determined by direct plating on Levine’s Eosin Methylene Blue agar medium (L-EMB) from both serial dilutions of the broth and yoghurt samples at the time of inoculation (Gulmez and Guven, 2003). The infective dose of *E. coli* ranges between 10-10⁷ cells (Mamajoro, 2009). In our study we used higher inoculum of the microorganism (6.5×10⁷ CFU/mL), because these numbers are high enough to cause food poisoning.

**Reference strain of *Listeria monocytogenes***: The organism was inoculated in tryptic soya broth with 0.6% yeast extract, incubated at 37°C for 24 h, then tenth fold serial dilution was made, inoculation level was determined by direct plating on oxford agar medium from both serial dilutions of the broth and yoghurt samples at the time of inoculation (Gulmez and Guven, 2003). Inoculation level of *L. monocytogenes* was 3×10⁷ CFU/mL.

**Yoghurt starter culture YC-380 (High viscosity medium flavor):** Lyophilized culture for Direct Vat Set (DVS) type *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* (YC-380) were used (Chr. Hansen Laboratories, Copenhagen, Denmark). The fermented culture was used according to the manufacturer’s description.

**Experimental technique:** Yoghurt was prepared in the laboratory according to the procedure described by Hui (1992).

For each pathogenic micro-organism, 1 L of the received raw milk was heated to 85°C for 30 min, then cooled immediately in an ice bath to inoculation temperature of 42°C, the exact amount of starter was added according to the manufacturer’s description followed by stirring. The
inoculated milk was divided into two parts, the first part was inoculated with the selected pathogenic microorganisms, followed by stirring while the second part was left as control. The inoculated milk was distributed into sterile small plastic cups (Euro tubes 50 mL capacity) and incubated at 42°C for 5-6 h in a thermostatically controlled water bath (Precision scientific, Chicago, USA). After complete fermentation, samples were examined as zero time and the remained cups were transferred to the refrigerator to be stored at 4°C and examined microbiologically every 3 days up to 15 days.

**Microbiological examination:**

- Determination of titratable acidity percentage and pH was done according to APHA (2004)
- Preparation of food homogenate and decimal dilutions according to APHA (2004)
- Enumeration of *Escherichia coli* using spreading technique according to Hutchins *et al.* (1992)
- Enumeration of *L. monocytogenes* using spreading technique according to ISO (2004)
- Enumeration of starter culture according to ISO (2003)

**Statistical analysis:** The experiment was carried out on triplicates and the average result was calculated and recorded.

**RESULTS AND DISCUSSION**

The survival of food borne pathogens for up to several days or weeks in fermented dairy products, specifically yoghurt, illustrates the potential health risks associated with post-processing contamination of these pathogens in various dairy products and there is a need for investigation of the survival period of these food borne pathogens in yoghurt before the finished product reaches the retail market, in order to heighten the awareness of post-processing contamination in the dairy industry (Dineen *et al.*, 1998). The fermentation temperature, type of fermentative micro-organisms, acid adaptation, acid tolerance and type of the pathogenic micro-organism play an important role on the survival of pathogenic micro-organisms in fermented dairy food (Pitt *et al.*, 2000).

**Influence of starter culture on viability of *Escherichia coli* (ATCC No. 25922) during storage of lab. manufactured yoghurt:** Data reported in Table 1 revealed the performance of starter culture and survival of *E. coli* (ATCC No. 25922) during the storage period at 4°C for

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Titratable acidity (%)</th>
<th>pH</th>
<th>E.C.C</th>
<th>S.T.C</th>
<th>L.B.C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>0.14</td>
<td>0.15</td>
<td>6.70</td>
<td>6.63</td>
<td>-</td>
</tr>
<tr>
<td>Zero* time</td>
<td>0.68</td>
<td>0.68</td>
<td>4.66</td>
<td>4.96</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0.77</td>
<td>0.75</td>
<td>4.37</td>
<td>4.46</td>
<td>4.9×10³</td>
</tr>
<tr>
<td>6</td>
<td>0.80</td>
<td>0.82</td>
<td>4.32</td>
<td>4.35</td>
<td>1.1×10³</td>
</tr>
<tr>
<td>9</td>
<td>0.86</td>
<td>0.90</td>
<td>4.25</td>
<td>4.30</td>
<td>2.4×10³</td>
</tr>
</tbody>
</table>

*After complete coagulation of milk, E.C.C: *E. coli* count; T: test, C: Control, S.T.C: Streptococcus salivarius ssp. thermophilus count, L.B.C: Lactobacillus delbrueckii ssp. bulgaricus count.
15 days, the titratable acidity percentage and pH value in bulk milk used for the experiment were 0.15% and 6.63, respectively, when the total initial count of E. coli was 6.5×10⁷ CFU/mL. By the end of the fermentation period the titratable acidity% increased to 0.68% and pH value dropped to 4.56, as a result of increasing the starter culture (Streptococcus salivarius ssp. thermophilus and Lactobacillus dulbruekii ssp. bulgaricus) count to 20.9×10⁷ and 100.0 CFU/mL, respectively, while the inoculated E. coli had a little increase in the count to 8×10⁷ CFU/mL, on the 6th day of storage there was a continuously increase in titratable acidity percentage to 0.82% and a little decrease in pH to 4.32, when the starter culture (Streptococcus salivarius ssp. thermophilus and Lactobacillus dulbruekii ssp. bulgaricus) count was 7.2×10⁶ and 20.0 CFU/mL, respectively, at the same time the inoculated E. coli had an immense drop in the count to 11×10⁵ CFU/mL. By the end of the ninth day the inoculated E. coli couldn’t be detected in the samples and this occurs when the starter culture (Streptococcus salivarius ssp. thermophilus and Lactobacillus dulbruekii ssp. bulgaricus) count, titratable acidity percentage and pH were 11.4×10⁶, 60.0 CFU g⁻¹, 0.82% and 4.35, respectively.

Nearly similar findings were obtained by Massa et al. (1997) and Al-Kadamany et al. (2003). On the other hand Canganella et al. (1998) found that at 4°C E. coli strains exhibited a higher tolerance to the yoghurt environment. Cells were still detectable in the samples after 21 days of storage, Guraya et al. (1998) found that the organism could survive for 14 day of yoghurt storage, also Benkerroum et al. (2002) could detect E. coli for up to 11 days in plain yoghurt and Bachrouri et al. (2006) who observed that E. coli survived in yoghurt for 20 days and could not be found at day 21 when stored at 4°C.

Acid tolerance of E. coli is a general characteristic shared by many enteric bacteria such as E. coli ATCC 25922 and its acid adaptation can enhance the survival of this organism in acidic dairy foods during fermentation (Gahan et al., 1996; Yi and Chou, 2001).

Influence of starter culture (YC-380) on viability of Listeria monocytogenes during storage of lab manufactured yoghurt: Low pH fermented dairy products, with strong antimicrobial activity of lysozyme and other metabolites have been reported to pose minor inhibiting effect on growth of several strains of L. monocytogenes (Griffith and Deibel, 1989). The pathogen can survive from 1-12 days during refrigerated storage of yoghurt, because the casein in yoghurt exerts a protective effect that L. monocytogenes was able to survive in yoghurt (Schaak and Marth, 1988).

Data depicted in Table 2 illustrated the effect of starter culture and the continued existence of L. monocytogenes during the storage period at 4°C for 15 days, after complete coagulation of milk,

Table 2: Influence of starter culture on viability of L. monocytogenes during the storage period of lab manufactured yoghurt

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Titratable acidity (%)</th>
<th>pH</th>
<th>L.M.C</th>
<th>S.T.C</th>
<th>L.B.C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>Milk</td>
<td>0.150</td>
<td>0.15</td>
<td>6.63</td>
<td>6.63</td>
<td>-</td>
</tr>
<tr>
<td>Zero⁰ time</td>
<td>0.840</td>
<td>0.83</td>
<td>4.21</td>
<td>4.29</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.550</td>
<td>1.14</td>
<td>4.05</td>
<td>4.10</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1.285</td>
<td>1.29</td>
<td>3.87</td>
<td>3.95</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>1.250</td>
<td>1.10</td>
<td>3.87</td>
<td>3.98</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>1.270</td>
<td>1.36</td>
<td>3.91</td>
<td>3.86</td>
<td>-</td>
</tr>
</tbody>
</table>

L.M.C: Listeria monocytogenes count.
the titratable acidity percentage increased from 0.15-0.83% and the pH value dropped from 6.68-4.29, as a result of increasing the starter culture (Streptococcus salivarius ssp. thermophilus and Lactobacillus delbrueckii ssp. bulgaricus) count to 7.9×10⁸, 15.0 CFU/mL, respectively, while the total L. monocytogenes count had a little decrease from 3×10⁴ to 2×10⁵ CFU/mL on the ninth day, the inoculated L. monocytogenes was dramatically dropped to 22×10³ CFU/mL, when the titratable acidity percentage was significantly increased to 1.1% and the pH value radically decreased to 3.98, when the starter culture (Streptococcus salivarius ssp. thermophilus) count was 4.7×10¹¹. The inoculated L. monocytogenes couldn’t be detected in yoghurt samples on the 12th day, as the titratable acidity% reached 1.36% and the pH value noticeably decreased as low as 3.86, when the starter culture (Streptococcus salivarius sub sp. thermophilus) count was 55×10¹².

Nearly similar results were obtained by Cottin et al. (1990), Massa et al. (1991) and Akkaya et al. (2009). On contrast Ashenañ (1994) mentioned that a substantial number of L. monocytogenes strains still survived even though the pH had markedly decreased to as low as 3.9.

From the obtained data we can conclude that the inoculated pathogenic microorganisms completely disappeared toward the end of the storage period, which may be associated with the decrease of pH below 4.0 (Zuniga-Estrada et al., 1995; Gahan et al., 1996). Enhancing the awareness of post-processing contamination in the dairy industry is of a major concern to reduce the incidence of entry of the pathogenic microorganisms (Dineen et al., 1998).

CONCLUSION
The results revealed that E. coli and L. monocytogenes can survive and multiply during the storage period of yoghurt, since these pathogens are resistant to acidic conditions. To prevent the food borne diseases caused by contaminated yoghurt, strict preventive measures should be taken reaching from the dairy farm to the manufacturing unit.

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