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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 6.5×10^7 and 3×10^7 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 Europan 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; Schaak 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 10-10^7 cells (Mamajoro, 2009). In our study we used higher inoculum of the microorganism (6.5×10^7 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 30°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 3×10^7 CFU/mL.

Yoghurt starter culture YC-380 (High viscosity medium flavor): Lyophilized culture for Direct Vat Set (DVS) type *Lactobacillus delbrueekii* 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 1: Influence of starter culture on viability of *E. coli* during the storage of lab manufactured yoghurt

	Titratable acidity (%)		pH		E.C.C		S.T.C		L.B.C	
Incubation period (days)	C	T	C	T	C	T	C	T	C	T
Milk	0.14	0.15	6.70	6.63	-	6.5×10^{7}	-	-	-	-
Zero* time	0.68	0.68	4.66	4.56	-	8.0×10^{7}	16.5×10^{6}	20.9×10^{7}	9×10^{2}	100.0
3	0.77	0.75	4.37	4.46	-	4.9×10^{4}	10.5×10^{10}	15.7×10^{9}	I20.0	120.0
6	0.80	0.82	4.32	4.35	-	11×10^{3}	18.0×10^{10}	7.2×10^{10}	21×10	20.0
9	0.86	0.90	4.25	4.30	-	-	24.0×10^{10}	11.4×10^{10}	26×10	60.0

*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 dulbrueekii* 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 6.5×10^7 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 dulbrueekii* ssp. *bulgaricus*) count to 20.9×10^7 and 100.0 CFU/mL, respectively, while the inoculated *E. coli* had a little increase in the count to 8×10^7 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 dulbrueekii* ssp. *bulgaricus*) count was 7.2×10^{10} and 20.0 CFU/mL, respectively, at the same time the inoculated *E. coli* had an immense drop in the count to 11×10^3 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 dulbrueekii* ssp. *bulgaricus*) count, titratable acidity percentage and pH were 11.4×10^{10}, 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 from1-12 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 of lab manufactured yoghurt

Incubation	Titratable acidity (%)		pH		L.M.C		S.T.C		L.B.C	
period (days)	C	T	C	T	C	T	C	T	C	T
Milk	0.150	0.15	6.63	6.63	-	3.0×10^7	-	-	-	-
Zero* time	0.840	0.83	4.21	4.29	-	2.0×10^7	5.1×10^9	7.90×10^9	140.0	15
3	1.150	1.14	4.05	4.10	-	8.5×10^5	1.9×10^{11}	17.2×10^{11}	65.0	25
6	1.285	1.20	3.87	3.95	-	3.5×10^4	5.7×10^{12}	6.90×10^{12}	40.0	25
9	1.290	1.10	3.87	3.98	-	22×10^3	4.6×10^{12}	4.70×10^{11}	15.0	-
12	1.270	1.36	3.91	3.86	-	-	5.2×10^{12}	55.0×10^{12}	15.0	-

L.M.C: *Listeria monocytogenes* count

the titratable acidity percentage increased from 0.15-0.83% and the pH value dropped from 6.63-4.29, as a result of increasing the starter culture (*Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus dulbrueekii* ssp. *bulgaricus*) count to 7.9×10^{9}, 15.0 CFU/mL, respectively while the total *L. monocytogenes* count had a little decrease from 3×10^{7}-2×10^{7} CFU/mL, on the ninth day, the inoculated *L. monocytogenes* was dramatically dropped to 22×10^{3} 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 4.7×10^{11}. 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 55×10^{12}.

Nearly similar results were obtained by Cottin *et al.* (1990), Massa *et al.* (1991) and Akkaya *et al.* (2009). On contrast Ashenafi (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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