JOURNAL OF FOOD QUALITY & HAZARDS CONTROL

SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 2 (2015) 122-127

Survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in Doogh, A Traditional Iranian Dairy Beverage

M. Zarei*, A. Fazlara, M. Pourmahdi Borujeni, M. Karimi

Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Article type
Original article

Keywords
Escherichia coli O157
Listeria monocytogenes
Dairy Products
Survival

Received: 28 Jul 2015
Revised: 30 Sep 2015
Accepted: 2 Nov 2015

Abstract

Background: Unlike industrially production, Iranian traditional doogh are not pasteurized after production. Hence, possible contamination with different pathogenic bacteria may occur during production or post-production of traditional doogh. The aim of the present study was to monitor the behavior of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in traditional Iranian doogh at different temperatures and time periods.
Methods: Low acid and high acid doogh samples were inoculated with approximately 4 and 6 log cfu/ml of each pathogen separately and then incubated at 4 °C and 25 °C. At different time interval, samples were taken to enumerate *E. coli* O157:H7 and *L. monocytogenes* and determine titratable acidity. Results were analyzed by ANOVA test using SPSS software (v. 16.0).
Results: Although the survival ability of *E. coli* O157:H7 in doogh samples was slightly higher than *L. monocytogenes*, both of them were detected in low acid doogh sample after 10-14 days at 4 °C. However, they were not detected in low acid doogh samples for more than 2 days at 25 °C. In contrast, in high acid doogh samples, the viability of *E. coli* O157:H7 and *L. monocytogenes* declined more quickly to undetectable level, both at 4 °C and 25 °C indicating less viability of these bacteria in high acid doogh samples compared to those of low acid ones ($p<0.05$).
Conclusion: Traditional Iranian doogh should be considered as a potential vehicle of transmission of *E. coli* O157:H7 and *L. monocytogenes*, especially in low acid doogh samples when stored under refrigeration.

Introduction

Interested in fermented dairy products is constantly increasing due to their microbiological and nutritional quality and the health promoting potential. Low pH and the lactic bacteria activity during fermentation could inactivate human pathogenic microorganisms in fermented dairy products (McKinley, 2005; Tamime, 2002). Doogh is a traditional Iranian drink, prepared by dilution of low fat yoghurt with water and further fermentation to achieve satisfactory taste and acidity, or occasionally by bacterial fermentation of milk with yoghurt culture; with the addition of salt and flavoring. It is one of many acidified dairy beverages produced worldwide which may differ from them in, for example, acidity, fat and salt content, dilution ratio, rheological characteristics, and taste (Azarikia and Abbasi 2010; Kiani et al., 2008). As a fermented dairy drink, consumption of doogh is very common in Iran especially during warm seasons. In addition to industrial production, this beverage is traditionally produced and consumed in various cities across the country. Unlike industrially production, those

*Corresponding author
E-mail: zarei@scu.ac.ir

traditionally produced are not pasteurized after production (Azarikia and Abbasi, 2010). Hence, possible contamination with different pathogenic bacteria may occur during production or post-production of traditional doogh.

Several authors have demonstrated that *Escherichia coli* O157:H7 and *Listeria monocytogenes* can survive in fermented dairy products over several days and weeks. (Bachrouri et al., 2002; Lekkas et al., 2006; Morgan et al., 2001; Ogwaro et al., 2002; Rogga et al., 2005; Simsek et al., 2007). The ability of these pathogens to survive and grow under various adverse environmental conditions, including high salt concentration and low pH, makes them potential hazards after the consumption of milk and dairy products (Cataldo et al., 2007; Ogwaro et al., 2002; Tienungoon et al., 2000; Vernozy-Rozand et al., 2005). Contamination of dairy products occurs as a result of either the use of contaminated raw milk or crosses contamination during and after processing (De Buyser et al., 2001; Lekkas et al., 2006; Rogga et al., 2005). Therefore, the fermented dairy products should be considered as potential route of transmission of *E. coli* O157:H7 and *L. monocytogenes*.

While the behavior of *E. coli* O157:H7 as well as *L. monocytogenes* has been extensively investigated in acidified dairy foods such as yoghurt and cheese, to the best of our knowledge, there is a lack of data regarding the survival of these pathogenic microorganisms in doogh. Hence, the main aim of the present investigation was to monitor the behavior of *E. coli* O157:H7 and *L. monocytogenes* in traditional Iranian doogh at different temperatures and time periods.

Materials and methods

Production of doogh samples

Low acid (0.6% lactic acid, LA) and high acid (0.9% lactic acid, HA) doogh samples were prepared by dilution (50:50) of LA and HA yoghurts with sterilized 2% sodium chloride solution and agitated vigorously for 5 min. Fat, protein and dry solids of the doogh samples were determined according to the standard methods (Wehr and Frank, 2004). The final LA and HA doogh samples contained 1% salt and approximately 0.7% fat, 1.7% protein and 4.9% dry solids.

Bacterial strains

Stock cultures of *E. coli* O157:H7 (ATCC 43895) and *L. monocytogenes* (ATCC 7644) were first activated by two successive transfers in tryptic soy broth (TSB) at 35 °C for 24 h. These activated cultures were served as the inoculum.

Preparation of the treatments

Stock cultures of each pathogen were inoculated into TSB and incubated at 35 °C for 20 h. After two successive cultures, the activated cultures of each pathogen were used for preparation of the treatments. Using appropriate dilutions of exactly 20 h bacterial cultures in TSB, approximately 4 and 6 log cfu/ml of each pathogen were inoculated into 50 ml of LA and HA doogh samples, separately. Samples were incubated at 4 °C and 25 °C and at different time intervals (at 4 °C every two days and at 25 °C every day). After that, the samples were taken to enumerate *E. coli* O157:H7 as well as *L. monocytogenes* and determine titratable acidity.

Microbiological analyses

Decimal dilutions of doogh samples were prepared in 0.85% (w/v) sterilized sodium chloride (NaCl) solution and surface plated (from undiluted sample to 10^{-3} for the lower inoculum level and to 10^{-5} for the higher inoculum level) on appropriate media. Tryptic soy agar (TSA) containing 100 µg/ml ceftazidime was used for the enumeration of *E. coli* O157:H7 and *L. monocytogenes* in doogh samples. Ceftazidime was used to prevent the growth of yoghurt's starter cultures on TSA. This antibiotic had no inhibitory effect on *E. coli* O157:H7 and *L. monocytogenes* (data not shown). Plates were incubated at 35 °C for 24-36 h, and then counted for viable organisms (APHA, 2001).

Determination of titratable acidity

Titratable acidity of the samples were determined by titrating 20 ml of doogh samples with 0.1 N NaOH solutions and expressed as percent of lactic acid.

Statistical analysis

All experiments were performed in triplicate. Data were analyzed using the repeated measures ANOVA by SPSS software (Chicago, IL, v. 16.0). The significance levels are expressed at a 95% confidence level ($p<0.05$) throughout.

Results

The behavior of *E. coli* O157:H7 in LA and HA doogh samples during storage at 4 °C is presented in Fig. 1 and Fig. 2. As shown, when the initial count was approximately 4 log cfu/ml (Fig. 1), count of *E. coli* O157:H7 was declined sharply to undetectable level in HA doogh samples. While in HA doogh samples, *E. coli* O157:H7 was not detected at the 4th day of storage, in LA doogh samples, *E. coli* O157:H7 survived for a longer period ($p<0.05$). In LA doogh samples, the viable cells of *E. coli*

O157:H7 were decreased gradually during the first 6 days of storage and reached to 3.65 ± 0.12 log cfu/ml. After that, the viable cells were declined more rapidly and finally, *E. coli* O157:H7 was not detected at the 12th day of storage. When the initial count was approximately 6 log cfu/ml (Fig. 2), *E. coli* O157:H7 was detected for a longer period in both LA and HA doogh samples, compared to the initial count of 4 log cfu/ml. In this case, in LA doogh samples, the viable cells of *E. coli* O157:H7 was decreased gradually until the 14th day of storage, where it was not detected anymore. However, in HA doogh samples the rate of the reduction of viable cells was faster and *E. coli* O157:H7 was not detected at the 6th day of storage (p<0.05). Regardless of the level of the initial count, during the storage period at 4 $^{\circ}$C, the percent of lactic acid in LA and HA doogh samples increased from 0.60% to 0.66% and from 0.90% to 0.92%, respectively.

Significant statistical differences were observed in the survival behavior of *E. coli* O157:H7 between 4 $^{\circ}$C and 25 $^{\circ}$C (p<0.05). As shown in Fig. 3 and Fig. 4, when doogh samples stored at 25 $^{\circ}$C, regardless of the initial *E. coli* O157:H7 count, the viable population of *E. coli* O157:H7 in LA and HA doogh samples declined quickly; where, on the 3rd day of storage at 25 $^{\circ}$C, no viable cells were detected in LA and HA doogh samples. Regardless of the level of the initial count, during the storage period at 25 $^{\circ}$C, the percent of lactic acid in LA and HA doogh samples increased from 0.60% to 0.76% and from 0.90% to 0.97%, respectively.

The behavior of *L. monocytogenes* in LA and HA doogh samples during storage at 4 $^{\circ}$C is illustrated in Fig. 5 and Fig. 6. As indicated, when the initial count was approximately 4 log cfu/ml (Fig. 5), count of *L. monocytogenes* was declined sharply to undetectable level in HA doogh samples. While in HA doogh samples, *L. monocytogenes* was not detected at the 4th day of storage; in LA doogh samples, *L. monocytogenes* survived for a longer period and the viable cells of *L. monocytogenes* were decreased gradually until the 10th day of storage, where it was not detected anymore (p<0.05). When the initial count was approximately 6 log cfu/ml (Fig. 6), *L. monocytogenes* was detected for a longer period in LA doogh samples, compared to the initial count of 4 log cfu/ml. In this case, the viable cells of *L. monocytogenes* were decreased gradually until the 12th day of storage, where it was not detected anymore. However, in HA doogh samples the rate of the reduction of viable cells was faster and *L. monocytogenes* was not detected at the 4th day of storage (p<0.05).

Significant statistical differences were observed in the survival behavior of *L. monocytogenes* between 4 $^{\circ}$C and 25 $^{\circ}$C (p<0.05). According to Fig. 7 and Fig. 8, when doogh samples inoculated with 4 log cfu/ml and stored at 25 $^{\circ}$C (Fig. 7), after 2 and 3 days of storage no viable cells were recovered from HA and LA doogh samples, respectively. Almost the same survival pattern was observed when 6 log cfu/ml inoculated into the LA and HA doogh samples (Fig. 8). In this case, on the 3rd dat of storage at 25 $^{\circ}$C, no viable cells were detected in LA and HA doogh samples.

Finally, comparing the survival behavior of *E. coli* O157:H7 and *L. monocytogenes* in LA and HA doogh samples indicated that *E. coli* O157:H7 survived for a longer period than *L. monocytogenes* at 4 $^{\circ}$C with a significant difference (p<0.05).

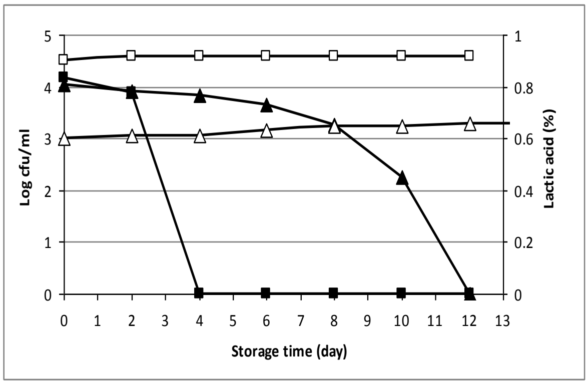

Fig. 1: Survival of *E. coli* O157:H7 in LA and HA doogh samples during storage at 4 $^{\circ}$C with initial counts of 4 log cfu/ml. ▲: count in LA doogh; ■: count in HA doogh; Δ: acidity in LA doogh; □: acidity in HA doogh

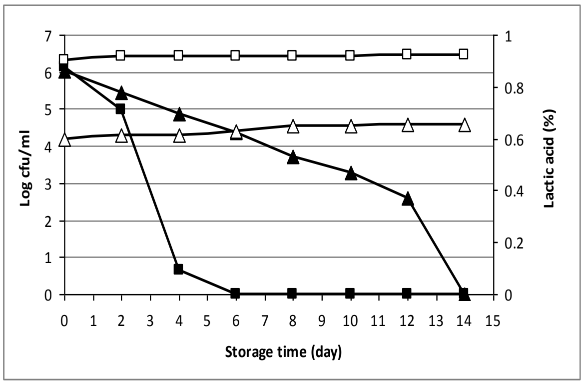

Fig. 2: Survival of *E. coli* O157:H7 in LA and HA doogh samples during storage at 4 $^{\circ}$C with initial counts of 6 log cfu/ml. ▲: count in LA doogh; ■: count in HA doogh; Δ: acidity in LA doogh; □: acidity in HA doogh

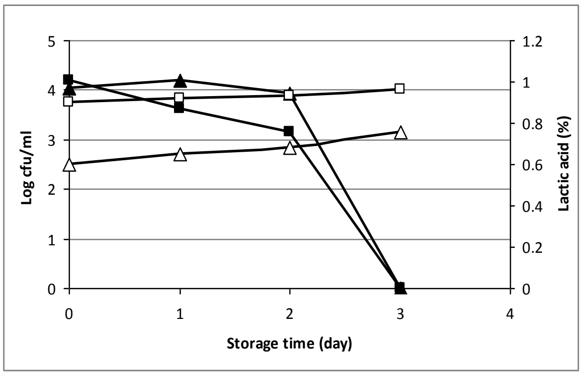

Fig. 3: Survival of *E. coli* O157:H7 in LA and HA doogh samples during storage at 25 °C with initial counts of 4 log cfu/ml. ▲: count in LA doogh; ■: count in HA doogh; Δ: acidity in LA doogh; □: acidity in HA doogh

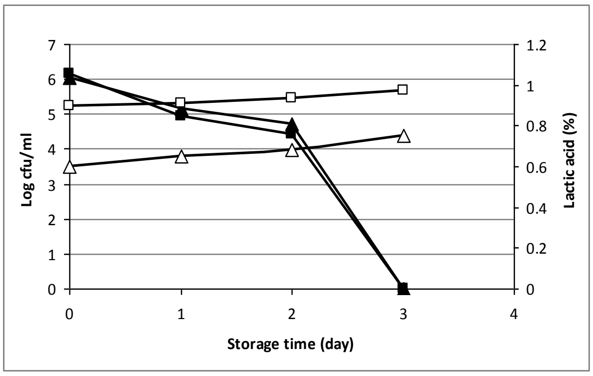

Fig. 4: Survival of *E. coli* O157:H7 in LA and HA doogh samples during storage at 25 °C with initial counts of 6 log cfu/ml. ▲: count in LA doogh; ■: count in HA doogh; Δ: acidity in LA doogh; □: acidity in HA doogh

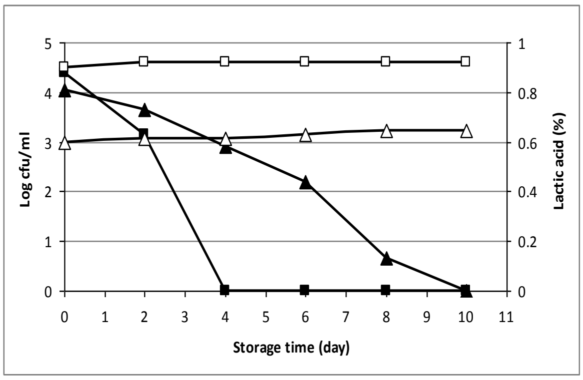

Fig. 5: Survival of *L. monocytogenes* in LA and HA doogh samples during storage at 4 °C with initial counts of 4 log cfu/ml. ▲: count in LA doogh; ■: count in HA doogh; Δ: acidity in LA doogh; □: acidity in HA doogh

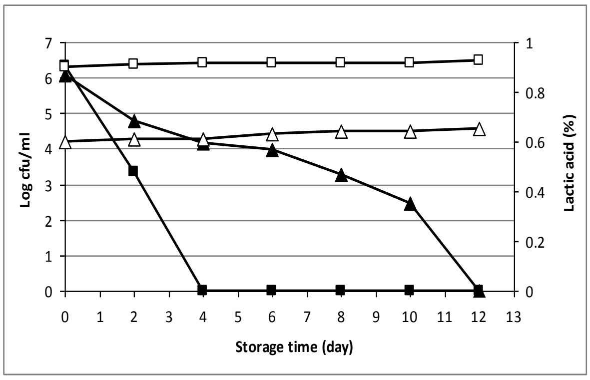

Fig. 6: Survival of *L. monocytogenes* in LA and HA doogh samples during storage at 4 °C with initial counts of 6 log cfu/ml. ▲: count in LA doogh; ■: count in HA doogh; Δ: acidity in LA doogh; □: acidity in HA doogh

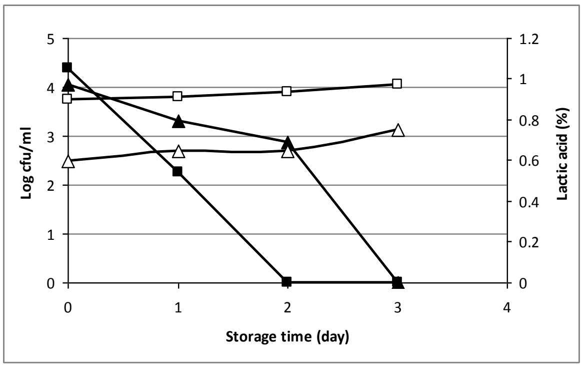

Fig. 7: Survival of *L. monocytogenes* in LA and HA doogh samples during storage at 25 °C with initial counts of 4 log cfu/ml. ▲: count in LA doogh; ■: count in HA doogh; Δ: acidity in LA doogh; □: acidity in HA doogh

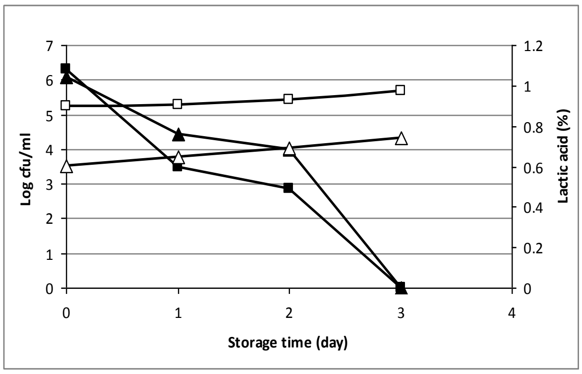

Fig. 8: Survival of *L. monocytogenes* in LA and HA doogh samples during storage at 25 °C with initial counts of 6 log cfu/ml. ▲: count in LA doogh; ■: count in HA doogh; Δ: acidity in LA doogh; □: acidity in HA doogh

Discussion

The behavior of *E. coli* O157:H7 and *L. monocytogenes* in traditional Iranian doogh at different temperatures and time periods was assessed in the present study. Results indicated that, both *E. coli* O157:H7 and *L. monocytogenes* were capable to survive in traditional Iranian doogh, despite the acidity of the product. As expected, both pathogens survived for significantly longer period in LA doogh samples compared to HA doogh samples. The ability of these pathogens for survival in acidic dairy products has been reported previously. Simsek et al. (2007) evaluated the behavior of *E. coli* O157:H7 during the storage of Ayran (a Turkish fermented milk beverage that is very similar to doogh) and reported that *E. coli* O157:H7 was detected in Ayran samples until 14 days of storage at 4 °C. Furthermore, Morgan et al. (2001) examined the survival of *L. monocytogenes* during manufacture, ripening and storage of soft lactic cheese made from raw goat milk. They showed that the physico-chemical and microbiological characteristics of lactic cheeses caused a decrease of *L. monocytogenes* counts. However, this reduction did not cause the complete disappearance of the pathogen and *L. monocytogenes* was able to survive in soft lactic cheeses made with raw goat milk until 42 days. Rogga et al. (2005) evaluated the survival of *L. monocytogenes* in fresh Galotyri cheese during storage at 4 °C and 12 °C. Their results indicated that *L. monocytogenes* could survive during retail storage of Galotyri cheese despite its low pH, where *L. monocytogenes* was found in all samples after 28 days of storage at 4 °C and 14 days of storage at 12 °C. Furthermore, *E. coli* O157:H7 were found in raw goat milk lactic cheeses throughout processing, and even after 42 days of ripening (Vernozy-Rozand et al., 2005).

According to the results of this study, the behavior of both pathogens in doogh samples was influenced by the storage temperature, the inoculum level and the amount of acidity. Survival of both pathogens was significantly longer at 4 °C than at 25 °C. It has been shown previously that, inactivation of pathogenic bacteria in acidic foods, including dairy products was enhanced at ambient temperature as compared to refrigerated storage conditions, due to an accelerating effect of the higher temperatures on the killing effect of acids (Bachrouri et al., 2002; Getty et al., 2000; Hsin-Yi and Chou, 2001). In Amasi (a traditional fermented milk product consumed in South Africa), *E. coli* O157:H7 was detectable in commercial Amasi after 3 days at 7 °C but not in traditional Amasi processed at ambient temperature over the same period (Dlamini and Buys, 2009). The same results have been reported by Ogwaro et al. (2002) in a traditional African yoghurt. They reported that, the viable count of *E. coli* O157:H7 inoculated post-fermentation into yoghurt samples did not decrease a lot during 6 days of storage at 4 °C, however, it was decreased to non-detectable level during the same period of storage at 25 °C.

Furthermore, LA resistance to *L. monocytogenes* compared to *E. coli* O157:H7 which was observed in this study, has been reported in other studies as well. For example, Gulmez and Guven (2003) examined the survival of *E. coli* O157:H7, *L. monocytogenes*, and *Yersinia enterocolitica* in yoghurt and kefir samples. They inoculated these pathogens into yoghurt and kefir samples and detected a significant number of *E. coli* O157:H7 after 10 days of incubation at 4 °C. *L. monocytogenes* was also detected until the 10th day but in a lower number compared to *E. coli* O157:H7, however, *Y. enterocolitica* was not detected in yoghurt and kefir samples stored at 4 °C after 5 days of storage.

Conclusion

Survival of *E. coli* O157:H7 and *L. monocytogenes* in LA doogh samples for up to several days highlights the potential health risks associated with the consumption of traditional Iranian doogh. This is especially important when the product is stored at 4 °C, a temperature that will control the growth of these pathogens, but will facilitate survival of existing pathogens. In addition to storage temperature, the results showed that the higher the inoculum level, the longer the survival of these pathogens in LA doogh samples.

Conflicts of interest

The authors declare that they have no conflict of interest in this research.

Acknowledgements

This publication is the results of a Master thesis which was supported by the research grant provided by Shahid Chamran University of Ahvaz. The authors would like to thank Mrs. P. Esfahani for her kind assistance.

References

American Public Health Association (APHA). (2001). Compendium of methods for the microbiological examination of foods. 4th edition. Washington, DC: American Public Health Association.

Azarikia F., Abbasi S. (2010). On the stabilization mechanism of Doogh (Iranian yoghurt drink) by gum tragacanth. *Food Hydrocolloids*. 24: 358-363.

Bachrouri M., Quinto E.J., Mora M.T. (2002). Survival of *Escherichia coli* O157:H7 during storage of yoghurt at different temperatures. *Journal of Food Science*. 67: 1899-1903.

Cataldo G., Conte M.P., Chiarini F., Seganti L., Ammendolia M.G., Superti F., Longhi C. (2007). Acid adaptation and survival of

Listeria monocytogenes in Italian-style soft cheeses. *Journal of Applied Microbiology*. 103: 185-193.

De Buyser M.L., Dufour B., Maire M., Lafarge V. (2001). Implication of milk and milk products in food-borne diseases in France and different industrialized countries. *International Journal of Food Microbiology*. 67: 1-17.

Dlamini B.C., Buys E.M. (2009). Adaptation of *Escherichia coli* O157:H7 to acid in traditional and commercial goat milk Amasi. *Food Microbiology*. 26: 58-64.

Getty K.J., Phebus R.K., Marsden J.L., Fung D.Y., Kastner C.L. (2000). *Escherichia coli* O157:H7 and fermented sausages: a review. *Journal of Rapid Methods and Automation in Microbiology*. 8: 141-170.

Gulmez M., Guven A. (2003). Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes* 4b, and *Yersinia enterocolitica* O3 in different yogurt and kefir combinations as prefermentation contaminant. *Journal of Applied Microbiology*. 95: 631-636.

Hsin-Yi C., Chou C.C. (2001). Acid adaptation and temperature effect on the survival of *Escherichia coli* O157:H7 in acidic fruit juice and lactic fermented milk product. *International Journal of Food Microbiology*. 70: 189-195.

Kiani H., Mousavi S.M.A., Emam-Djomeh Z. (2008). Rheological properties of Iranian yoghurt drink, doogh. *International Journal of Dairy Science*. 1: 71-78.

Lekkas C., Kakouri A., Paleologos E., Voutsinas L.P., Kontominas M.G., Samelis J. (2006). Survival of *Escherichia coli* O157:H7 in Galotyri cheese stored at 4 and 12 °C. *Food Microbiology*. 23: 268-276.

McKinley M.C. (2005). The nutrition and health benefits of yoghurt. *International Journal of Dairy Technology*. 58: 1-12.

Morgan F., Bonnina V., Mallereau M.P., Perrin G. (2001). Survival of *Listeria monocytogenes* during manufacture, ripening and storage of soft lactic cheese made from raw goat milk. *International Journal of Food Microbiology*. 64: 217-221.

Ogwaro B.A., Gibson H., Whitehead M., Hill D.J. (2002). Survival of *Escherichia coli* O157:H7 in traditional African yoghurt fermentation. *International Journal of Food Microbiology*. 79: 105-112.

Rogga K.J., Samelis J., Kakouri A., Katsiari M.C., Savvaidis I.N., Kontomina M.G. (2005). Survival of *Listeria monocytogenes* in Galotyri, a traditional Greek soft acid-curd cheese, stored aerobically at 4 °C and 12 °C. *International Dairy Journal*. 15: 59-67.

Simsek B., Sagdic O., Ozcelik S. (2007). Survival of *Escherichia coli* O157:H7 during the storage of Ayran produced with different spices. *Journal of Food Engineering*. 78: 676-680.

Tamime A.Y. (2002). Fermented milks: a historical food with modern applications-a review. *European Journal of Clinical Nutrition*. 56: 2-15.

Tienungoon S., Ratkowsky D.A., McMeekin T.A., Ross T. (2000). Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. *Applied and Environmental Microbiology*. 66: 4979-4987.

Vernozy-Rozand C., Mazuy-Cruchaudet C., Bavai C., Montet M.P., Bonin V., Dernburg A., Richard Y. (2005). Growth and survival of *Escherichia coli* O157:H7 during the manufacture and ripening of raw goat milk lactic cheeses. *International Journal of Food Microbiology*. 105: 83-88.

Wehr H.M., Frank J.F. (2004). Standard methods for the examination of dairy products. 17th edition. Washington, DC: American Public Health Association.