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Survival of *Listeria monocytogenes* in Ayran, a traditional Turkish fermented drink

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Abstract

Ayran is a traditional fermented dairy product produced by mixing milk or yoghurt, water and salt. In this study, survival of *Listeria monocytogene*s 1/2b was investigated in Ayran samples. For that purpose, Ayran samples produced from yoghurt (Group A and B) or directly form milk (Group C and D) were contaminated with 1 % concentration of 7 (A1, B1, C1 and D1 samples) or 5 (A2, B2, C2 and D2 samples) log cfu/mL of *L. monocytogenes*. So, eight different samples of Ayran were produced and stored at 4 ºC (Group A and C) or 20 ºC (Group B and D) for 21 days. According to the obtained results, there was no significant change in the number of viable cells of *L. monocytogenes* 1/2b in samples A1, A2, C1 and C2 after 21 days of the storage period. However, *L. monocytogenes* 1/2b cells of B1 and B2 samples were completely inhibited after 5 days of storage at 20 ºC. Similarly to that, *L. monocytogenes* 1/2b cells in samples D1 and D2 were also completely inhibited after 3 days of the storage. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* viable cell counts were between 10^3-10^7 cfu/mL in all of the samples. Consequently, Ayran contaminated with *L. monocytogenes* may contribute to a risk for public health. But, due to low pH of approximately ≤3.90, Ayran is relatively safe from the risk of *L. monocytogenes*.

Key words: Ayran, *Listeria monocytogenes*, yogurt, survival

Introduction

Ayran is a fermented milk traditionally manufactured by mixing yoghurt, water and salt. Thanks to refreshing properties, Ayran continues to be one of the most popular drinks in Turkey (Oztabak, 1996; Simsek et al., 2007). Ayran is generally consumed during the summer months in Turkey (Koksoy and Kilic, 2003) and is usually sold in places such as dairies, food stands and restaurants. Current production technology of Ayran implies adjustment of the milk's dry matter before incubation and water addition methods to even out the coagulation forming after incubation or to smooth out the coagulation forming after manufacturing regular yogurt (Demirci and Simsek, 1997). The *Listeria* species is commonly found in the environment, soil, water, feed, animals, personals and equipment.

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Due to insufficient sanitation applications, food products may be contaminated during the production, transport and consuming processes by these sources (Bracket, 1988; Bahk and Marth, 1990). In current years Listeriosis, one of *Listeria* infections caused by contaminated foods, became more frequent and in some cases it can lead to mortalities (Bracket, 1988). *Listeria monocytogenes* can cause mastitis (Fleming et al., 1985) - the inflammation of animal breast tissue (Barza, 1985; Sanaa et al., 1993), thus contaminating milk and milk products from mastitis breast (Schlech et al., 1983; Linnan et al., 1988). There are numerous studies on *Listeria* contamination of yoghurt in various countries (Al-Shaikhli, 1980; Salji et al., 1987; Wessels et al., 1988; Tipparaju et al., 2004) including Turkey (Kaptan and Gursel, 1984; Ergun et al., 1990). According to Schaack and Marth (1988), in order to produce microbiologically safe and high quality fermented milk products, it is important to apply appropriate fermentation temperature, strict sanitation procedures and pasteurization norms, and to use an active thermophile starter culture.

Results of some studies carried out on the retailed Ayran in Turkey (Agaoglu et al., 1998; Gulmez et al., 2003) showed that the quality of hygiene was substandard. The purpose of this study was to reveal the behaviour of the microorganism *L. monocytogenes* in Ayran which was contaminated by scientific methods at different levels and how it is affected by various storage temperatures.

Materials and Methods

Determination of pH value

The pH value of the Ayran samples was measured by a pH meter (InoLab pH 720 model, Germany).

Listeria monocytogenes strain

Listeria monocytogenes 1/2b strain used in the study was obtained from Prof. M. P. Doyle (Center for Food Safety Quality Enrichment Dept. of Food Science Techn., The University of Georgia Griffin, Georgia, USA).

Inoculum preparation

L. monocytogenes 1/2b obtained from stock culture was grown in Nutrient broth and incubated at 37 °C for 18 h. Fresh broth culture was prepared overnight in a nutrient broth and later adjusted, so that the final concentration of each sample after inoculation was approximately 10^3 or 10^5 cfu/mL. *L. monocytogenes* inoculum was added to Ayran samples in two different ways. In the first way, milk was contaminated with *L. monocytogenes* after pasteurization. Then, yoghurt for Ayran production was produced from the contaminated milk. In the second way Ayran was produced directly from Ayran milk by adding 50 % sterile distilled water and 1 % salt and was separately contaminated by adding 10^5 or 10^7 cfu/mL of *L. monocytogenes* fresh culture.

Production of experimental Ayran samples

The 3 % of starter culture containing *Lb. delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (Chr. Hansens, YC-180) was inoculated into 3 L of milk, which was heated at 90 °C for 5 minutes and cooled down to 43 °C under laboratory conditions. After inoculation with the starter culture, the milk was divided into 3 parts.

The first part of the milk (1 L) was divided into two parts. Then, the milks were again distributed into two sterilized 500 mL glass jars and incubated at 43 °C until reaching pH 4.8. In each jar, the yogurt samples were cooled at 4 °C for 16 hours. Then 50 % sterile distilled water and 1 % salt were added to the yoghurt to prepare Ayran. Each Ayran sample was separately contaminated with 1 % of *L. monocytogenes* culture containing 5 or 7 log cfu/mL bacteria and stored for 21 days at temperatures of 4 °C or 20 °C to form four different experimental groups (A1: inoculated with 7 log cfu/mL of *L. monocytogenes* and stored at 4 °C; A2: inoculated with 5 log cfu/mL of *L. monocytogenes* and stored at 4 °C; B1: inoculated with 7 log cfu/mL of *L. monocytogenes* and stored at 20 °C; B2: inoculated with 5 log cfu/mL of *L. monocytogenes* and stored at 20 °C).

The second batch of milk (1 L) was added to 50 % sterilized distilled water and 1 % salt for direct Ayran production. Then, the inoculated milk was divided into four different sterilized 500 mL jars and the all jars were incubated at 43 °C until being pH

4.8. After incubation, the Ayran samples were cooled at 4 °C for 16 hours. The 1 % of *L. monocytogenes* 1/2b reference strain was separately inoculated into the Ayran samples in the jars at levels of 5 or 7 log cfu/mL and stored for 21 days at 4 °C or 20 °C to form four different experimental groups at this stage (C1: inoculated with 7 log cfu/mL of *L. monocytogenes* and stored at 4 °C; C2: inoculated with 5 log cfu/mL of *L. monocytogenes* and stored at 4 °C; D1: inoculated with 7 log cfu/mL of *L. monocytogenes* and stored at 20 °C; D2: inoculated with 5 log cfu/mL of *L. monocytogenes* and stored at 20 °C).

Additionally, third part of the milk was a control group (without L. monocytogenes). It was used in normal yogurt production and then in Ayran production from the yogurt added to 50 % sterile distilled water and 1 % salt. Thus 9 different experimental groups were formed with this work (A1, A2, B1, B2, C1, C2, D1, D2 and control).

Microbiological analyses

In order to analyse streptococci and lactobacilli, 10 g of Ayran sample was measured into each sterile stomacher bag after which 90 mL of sterile peptone physiologic serum (0.85 % NaCl + 0.1 % peptone) was added into each bag (Interscience, UK) and homogenized in the stomacher bags for 2 minutes. After diluting the samples at a scale of 1:10, decimal solutions were prepared up to 10^{-7}. The prepared dilutions were anaerobically cultured on MRS (de Man Rogosa Sharpe, MRS; Oxoid, Germany) at 42 °C for 2-3 days and M17 (Oxoid, Germany) at 42 °C for 2-3 days. After incubation, colonies of *Lactobacillus* on MRS Agar and colonies of S. thermophilus on M17 Agar were counted as colony forming unites (cfu) (Baumgart, 1993; Pichhardt, 1993).

The direct counting method was used to determine the number of *L. monocytogenes* in the Ayran samples experimentally inoculated with *L. monocytogenes*. The dilutions prepared up to 10^{-7} cfu/mL were enriched with LSA (Listeria Selective Agar + Listeria Selective Supplement, Oxoid, Germany) and enumerated using the drop plaque method after which they were left to incubate at 35 °C for 48 hours. At the end of the incubation period, typical colonies surrounded with black halos and 1-3 mm in diameter was evaluated as suspicious for *L. monocytogenes* (Curtis et al., 1989). After incubation, each of the *Listeria* suspicious colonies developing in each plate were enriched with Tryptic Soy Agar-Yeast Extract (Difco, Germany) for purification and after incubating at 30 °C for 24 hours the colonies were inspected morphologically and for purity by carrying out Gram staining. In the tests, colonies which proved Gram and catalase positive, oxidase negative, reproducing umbrella style in SIM medium were evaluated as *Listeria* spp. β-hemolysis in hematite agar, xylose, L-rhamnose, salicin, dulcite, methyl red, Voges Proskauer, nitrate reduction and CAMP tests were utilized to identify *Listeria* isolated from the samples (Baird et al., 1989; Curtis et al., 1989; Van Netten et al., 1989; Jemmi, 1990; Hidchins, 2002). Microbact™ TM 12L *Listeria* identification system (Oxoid, Germany) was used according to manufacturer's instructions to certify identified isolates.

At the 0, 1st, 3rd, 5th, 10th, 15th and 21st days of the storage, the number of *L. monocytogenes*, streptococci and lactobacilli in the trial Ayran were determined.

Isolation and identification of L. monocytogenes with enrichment method

The enrichment method was used to isolate and identify *L. monocytogenes* in the Ayran samples which were experimentally inoculated with *L. monocytogenes* although it could not be determined by direct counting method. 25 g of each analysed sample was taken and placed in sterile stomacher bags which were enriched with 225 mL of Listeria Enrichment Broth (LEB, Oxoid, Germany), after which the contents were homogenized in the stomacher bags (Bagmixer, Interscience) for 2 minutes and incubated in aerobe conditions at 30 °C for 24 hours. Following incubation 0.1 mL homogenized from the LEB was transferred into tubes containing 10 mL each Fraiser Broth (Oxoid, Germany) and incubated again at 30 °C for 24 hours. The 0.1 mL homogenized acquired after this procedure was drawn in to Palcam Agar (Oxoid, Germany) and Oxford Agar (Oxoid, Germany) and each plate was left to incubate at 30 °C for 48 hours.

Table 1. Microbiological characteristics and pH values of Ayran samples produced from yogurt and contaminated with L. monocytogenes

Samples	days	pH	*L. monocytogenes* (log cfu/mL)	*S. thermophilus* (log cfu/mL)	*Lb. delbrueckii* ssp. *bulgaricus* (log cfu/mL)
A1	0	6.95 ^{a}	5.17 ^{c}	6.57 ^{c}	6.57 ^{a}
	1	5.29 ^{c}	5.10 ^{d}	7.17 ^{a}	6.18 ^{c}
	3	5.19 ^{e}	4.02 ^{f}	6.80 ^{b}	6.43 ^{b}
	5	5.44 ^{b}	4.91 ^{e}	5.10 ^{e}	6.43 ^{b}
	10	5.30 ^{c}	5.3 2 ^{b}	5.27 ^{d}	6.63 ^{a}
	15	5.25 ^{d}	5.79 ^{a}	4.91 ^{f}	6.63 ^{a}
	21	5.13 ^{f}	5.10 ^{d}	4.72 ^{g}	5.32 ^{d}
A2	0	6.96 ^{a}	3.39 ^{a}	6.52 ^{a}	3.57 ^{e}
	1	5.59 ^{b}	3.19 ^{b}	3.79 ^{e}	6.90 ^{a}
	3	5.21 ^{f}	2.43 ^{c}	4.43 ^{b}	6.06 ^{d}
	5	5.41 ^{c}	2.02 ^{d}	3.43 ^{g}	6.06 ^{d}
	10	5.39 ^{d}	3.39 ^{a}	4.32 ^{c}	6.77 ^{b}
	15	5.27 ^{e}	3.34 ^{a}	4.04 ^{d}	6.36 ^{c}
	21	5.19 ^{g}	2.10 ^{d}	3.59 ^{f}	6.12 ^{d}
B1	0	6.96 ^{a}	5.17 ^{b}	6.57 ^{c}	6.57 ^{e}
	1	4.56 ^{b}	5.79 ^{a}	4.79 ^{f}	6.67 ^{d}
	3	3.92 ^{c}	<1	7.22 ^{a}	7.37 ^{b}
	5	3.86 ^{d}	ND	6.60 ^{b}	7.19 ^{c}
	10	3.84 ^{e}	ND	6.35 ^{e}	6.19 ^{f}
	15	3.80 ^{f}	ND	6.52 ^{d}	7.23 ^{c}
	21	3.80 ^{f}	ND	6.55 ^{d}	7.43 ^{a}
B2	0	6.96 ^{a}	3.39 ^{a}	6.52 ^{c}	3.57 ^{f}
	1	4.61 ^{b}	3.10 ^{b}	5.33 ^{e}	6.88 ^{e}
	3	3.92 ^{c}	<1	7.19 ^{a}	7.56 ^{b}
	5	3.88 ^{d}	ND	6.63 ^{b}	7.55 ^{b}
	10	3.85 ^{d}	ND	6.50 ^{c}	7.12 ^{d}
	15	3.81 ^{d}	ND	6.61 ^{b}	7.27 ^{c}
	21	3.81 ^{d}	ND	6.36 ^{d}	7.72 ^{a}

A1: Inoculated with 7 log cfu/mL of *L. monocytogenes* and stored at 4 °C, A2: Inoculated with 5 log cfu/mL of *L. monocytogenes* and stored at 4 °C, B1: Inoculated with 7 log cfu/mL of *L. monocytogenes* and stored at 20 °C, B2: Inoculated with 5 log cfu/mL of *L. monocytogenes* and stored at 20 °C, ND: Not detected, $^{a-h}$: Means in a same column with different letters are significantly different ($P<0.05$)

Statistical analysis

Statistical analysis of the data was performed using SPSS 15.0 Statistic Package (SPSS, Chicago, Illinois, USA). Statistical significance level was taken as 95 %. When analysis of variance (ANOVA) revealed a significant effect ($P<0.05$), the data means were compared by the least significant difference (Duncan's Multiple Range test) test.

Results and discussion

In control sample without *L. monocytogenes* was analyzed for *L. monocytogenes*, *Lb. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* counts. *L. monocytogenes* might not be found (<1 cfu/mL) in the control sample. In the same sample, *Lb. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* counts were 6.9 and 6.7 log cfu/mL, respectively.

Table 2. Microbiological characteristics and pH values of direct Ayran samples contaminated with *L. monocytogenes*

Samples	days	pH	*L. monocytogenes* (log cfu/mL)	*S. thermophilus* (log cfu/mL)	*Lb. delbrueckii* ssp. *bulgaricus* (log cfu/mL)
C1	0	3.99^{g}	5.16^{c}	6.36^{a}	5.79^{d}
	1	5.51^{a}	4.31^{d}	2.71^{e}	7.11^{a}
	3	5.24^{d}	4.32^{b}	4.61^{d}	6.81^{b}
	5	5.43^{b}	5.61^{d}	5.79^{c}	6.81^{b}
	10	5.37^{c}	5.79^{a}	5.81^{c}	5.61^{e}
	15	5.22^{e}	5.79^{a}	5.79^{c}	6.50^{c}
	21	5.11^{f}	5.11^{c}	6.09^{b}	6.40^{c}
C2	0	3.96	3.31^{a}	6.46^{a}	5.07^{e}
	1	5.48	3.11^{c}	3.78^{e}	7.13^{a}
	3	5.24	2.32^{f}	3.62^{e}	6.63^{c}
	5	5.51^{a}	2.62^{e}	3.18^{f}	6.63^{c}
	10	5.30^{c}	3.23^{b}	5.23^{b}	6.84^{b}
	15	5.19^{e}	2.79^{d}	5.11^{c}	6.40^{d}
	21	5.16^{f}	2.79^{d}	4.32^{d}	4.79^{f}
D1	0	3.98^{b}	5.16^{b}	6.36^{e}	5.79^{e}
	1	4.37^{a}	5.54^{a}	6.23^{f}	6.83^{d}
	3	3.89^{c}	<1	7.23^{a}	7.27^{a}
	5	3.83^{d}	ND	7.11^{b}	7.24^{a}
	10	3.82^{d}	ND	7.18^{a}	7.11^{b}
	15	3.81^{e}	ND	7.04^{c}	6.92^{c}
	21	3.79^{f}	ND	6.80^{d}	6.91^{c}
D2	0	3.97^{b}	3.31^{a}	6.46^{e}	5.07^{f}
	1	4.44^{a}	2.31^{b}	7.11^{c}	5.49^{e}
	3	3.91^{c}	ND	7.23^{b}	7.50^{a}
	5	3.79^{d}	ND	7.37^{a}	7.40^{b}
	10	3.78^{e}	ND	7.11^{c}	7.23^{c}
	15	3.76^{e}	ND	7.04^{c}	6.88^{d}
	21	3.76^{e}	ND	6.74^{d}	6.86^{d}

C1: Inoculated with 7 log cfu/mL of *L. monocytogenes* and stored at 4 °C, C2: Inoculated with 5 log cfu/mL of *L. monocytogenes* and stored at 4 ° C, D1: Inoculated with 7 log cfu/mL of *L. monocytogenes* and stored at 20 °C, D2: Inoculated with 5 log cfu/mL of *L. monocytogenes* and stored at 20 °C, ND: Not detected

$^{a-g}$: Means in a same column with different letters are significantly different ($P<0.05$)

In this study, while the pH levels of A1, A2, C1 and C2 samples were between 5.11 and 5.19 at the end of 21st day, reduction in *L. monocytogenes* levels were determined (Table 1, 2). Additionally, while the pH levels of B1, B2, D1 and D2 samples were between 3.79 and 3.86 at the end of 5th day, *L. monocytogenes* levels were completely inhibited at the end of this day (Table 1, 2). The pH values of Ayran samples coded as A1 and A2 were 5.13 and 5.19, *L. monocytogenes* counts of the same samples were 5.10 and 2.10 log cfu/mL at the end of the 21st day, respectively (Table 1). So, *L. monocytogenes* counts on the first and 21st day were similar to each other and *L. monocytogenes* survived in the Ayran samples. On the other hand, pH values of Ayran samples coded as B1 and B2 was 3.92 at the 3rd day of storage and viable *L. monocytogenes* cells of the samples were not enumerated by the direct count method (<1 log cfu/mL). After the pH levels of samples B1

and B2 reduced to 3.86 and 3.88 (at the end of 5th day) respectively, *L. monocytogenes* cells in the same samples were completely inhibited ($p<0.05$, Table 1). While C1 and C2 samples contaminated with 7 and 5 log cfu/mL of *L. monocytogenes* and stored at 4 °C had pH levels of 5.11 and 5.16 at the end of the 21st day, *L. monocytogenes* levels of the samples decreased to 5.11 and 2.79 log cfu/mL, respectively ($P<0.05$, Table 2). D1 sample contaminated with 7 log cfu/mL of *L. monocytogenes* and stored at 20 °C had no *L. monocytogenes* count estimated by the direct count method and had a pH value of 3.89 at the end of 3rd day. However, in 25 mL of sample D1 *L. monocytogenes* cells were observed by the pre-enrichment method at the end the 3rd day of storage, but not at the end of 5th day. At the 5th day, *L. monocytogenes* cells were completely lost in sample D1, and pH value was 3.83. Also, *L. monocytogenes* cells in sample D2 were completely inhibited at the 3rd day of the storage and pH value of 3.91.

Previous studies showed that 22 % of infections caused by *L. monocytogenes* originated from contaminated milk products (De Buyser et al., 2001). The growth of *L. monocytogenes* is mainly affected by factors such as temperature, pH, saline and antimicrobial agents (Doyle, 1988). *L. monocytogenes* as a psychrotrophic species is able to grow under refrigerator temperature and cause major public health problems (Walker et al., 1990). Fermentation and pH decrease below ≤5.2 affect the growth of *L. monocytogenes* and are able to inhibit its cells (Ryser, 1988). Additionally, lactic acid bacteria have generally inhibitory effect against pathogenic bacteria e.g. *L. monocytogenes* and the capability to produce acid and other metabolites including bacteriocins and H_2O_2 (Tipparaju et al., 2004).

Some researchers have studied the behaviour of *L. monocytogenes* in yoghurt during production and storage periods (Massa et al., 1991; Gulmez and Guven, 2003, Akkaya et al., 2009). Massa et al. (1991) investigated the survival of *L. monocytogenes* in yoghurt samples contaminated by addition of concentrations 10^3 cfu/mL. The observed results showed that viable cells of *L. monocytogenes* counts were only detectable by the pre-enrichment method during the 2nd day of the storage, while they were completely inhibited at the 5th day. Additionally, in yoghurt samples contaminated by addition 10^7 cfu/mL of *L. monocytogenes*, viable cells were determined by the pre-enrichment during the 7th day of the storage, and they were completely inhibited at the 15th day. Gulmez and Guven (2003) investigated yoghurt samples contaminated with 4.69 log cfu/mL of *L. monocytogenes* and stored for 10 days. While the pH value of the yoghurt was 4.2 at the end of fermentation, *L. monocytogenes* count decreased to approximately 3.0 log cfu/mL. Consequently, when at pH level 4.1 at the end of 10th day, the *L. monocytogenes* counts decreased to 0.6 log cfu/mL. Tipparaju et al. (2004) indicated that *L. monocytogenes* inoculated to fat or fat-free yogurt samples decreased from 7 log cfu/mL to 3 log cfu/mL at the end of 31st day of storage. In another study, *L. monocytogenes* was completely inhibited at the end of 7th day of the storage in strained yoghurt contaminated with 10^4 or 10^6 cfu/mL of *L. monocytogenes* (Akkaya et al., 2009). Our findings were similar to that of Akkaya et al. (2009).

As a result of the present study, it may be concluded that possible contamination of Ayran (stored at 4 °C) with *L. monocytogenes* before or after fermentation may represent a public health hazard. Sahin (2002) reported similar results to our findings. The viability of *L. monocytogenes* in Ayran is dependent upon inoculation amount, pH level, and storage temperature. In order to obtain microbiologically safe and high quality fermented milk products e.g. Ayran, it is important to apply appropriate fermentation temperature and strict sanitation procedures of milk, and to use thermophile starter culture. Additionally, GMP and HACCP systems must be applied to ensure the safety of Ayran for human consumption.

Preživljavanje bakterije Listeria monocytogenes u Ayranu, tradicionalnom turskom fermentiranom napitku

Sažetak

Ayran je tradicionalni fermentirani mliječni proizvod koji se dobiva miješanjem mlijeka ili jogurta, te vode i soli. U ovom je istraživanju ispitivano preživljavanje soja *Listeria monocytogenes* 1/2b u uzorcima Ayrana. U tu su svrhu proizvedeni uzorci Ayrana iz jogurta (skupina A i B) ili izravno iz mli-

jeka (skupina C i D) te kontaminirani dodatkom 1 % kulture soja *L. monocytogenes* 1/2b u koncentraciji 7 (uzorci A1, B1, C1 i D1) ili 5 (uzorci A2, B2, C2 i D2) log cfu/mL. Time je proizvedeno ukupno 8 različitih uzoraka Ayrana koji su čuvani 21 dan na 4 °C (skupina A i C) odnosno na 20 °C (skupina B i D). Dobiveni rezultati pokazali su da u uzorcima A1, A2, C1 i C2 broj živih stanica soja *L. monocytogenes* 1/2b nije bio promijenjen na završetku skladištenja. Međutim, stanice *L. monocytogenes* u uzorcima B1 i B2 bile su potpuno inhibirane na kraju petog dana skladištenja na 20 °C. Slično tomu, u uzorcima D1 i D2 su stanice dodane kulture soja *L. monocytogenes* 1/2b također bile inhibirane nakon tri dana skladištenja. Pritom je broj živih stanica jogurtne kulture sastavljene od vrsta *Streptococcus thermophilus* i *Lactobacillus delbrueckii* ssp. *bulgaricus* u svim uzorcima bio između 10^3-10^7 cfu/mL. Prema tome, Ayran kontaminiran bakterijom *L. monocytogenes* može uzrokovati rizik za zdravlje potrošača. Međutim, zbog relativno niskog pH koji se kreće ispod 3,90, Ayran je relativno siguran proizvod kad se radi o prisustvu bakterijske vrste *L. monocytogenes*.

Ključne riječi: Ayran, *Listeria monocytogenes*, jogurt, preživljavanje

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