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Research Article

Evaluation of Survival of *Salmonella* Typhimurium in Homemade Kefir

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Abstract

Kefir is a fermented probiotic dairy product which is fondly consumed with its peculiar, pleasant taste. However, like all dairy products, kefir may also constitute a risk for pathogenic microorganisms, which is particularly associated with its relatively long fermentation period that requires numerous processing steps. The objective of this study was to investigate the putative growth and risk status of *Salmonella* during the production and shelf life of kefir. For this purpose, kefir samples prepared with *Salmonella* reference culture-spiked milk, were divided into two groups; one was fermented at cold temperatures while the other was left to fermentation at ambient temperature. *Salmonella* enumeration and pH measurements of each group was performed at every 24 hours for six days. According to data obtained, *Salmonella* count has increased to 5.60 log cfu/ml on the first day of incubation at ambient temperature, which then started to decrease with ongoing fermentation. However, the bacteria was still present at the 6th day. Bacterial count of refrigerated kefir did not exhibit a sudden logarithmic rise, reaching its highest value at 2.87 log cfu/ml level and did not reveal a marked drop in comparison to the initial count, which instead sustained at 1.98 log cfu/ml level and thus bacteria survived until the end of the determined shelf life.

Keywords: *Kefir, Salmonella Typhimurium, Foodborne pathogens, Probiotic, Growth potential*

Ev Yapımı Kefirde *Salmonella* Typhimurium'un Canlılığının İncelenmesi

Özet

Kefir, probiyotik özelliği, hoş ve karakteristik lezzeti ile sevilerek tüketilen fermente süt ürünüdür. Buna karşın, tüm süt ürünlerinde olduğu gibi kefir de patojen mikroorganizma gelişimi açısından risk teşkil etmektedir. Özellikle, kefirin çok sayıda işlem gerektiren ve nispeten uzun olan fermantasyon aşaması bu riski akla getirmektedir. Bu çalışmada, önemli patojenlerden olan *Salmonella*'nın kefir üretimi ve raf ömrü boyunca gelişme durumunun ve risk seviyesinin incelenmesi amaçlanmıştır. Bunun için, referans *Salmonella* kültürü ile aşılanan sütlerden hazırlanan kefir örnekleri 2 gruba ayrılarak bir grubu soğuk muhafaza koşullarında, diğeri ortam sıcaklığında fermantasyona bırakılmıştır. Her iki grubun da 6 gün süresince 24 saatte bir *Salmonella* sayılarının tespiti ve pH ölçümleri yapılmıştır. Elde edilen sonuçlara göre ortam sıcaklığında inkübe edilen kefirde *Salmonella* sayısı inkübasyonun 1. gününde 5.60 log kob/ml seviyelerine kadar yükselmiş, daha sonra ilerleyen fermantasyonun da etkisi ile düşmeye başlamış, ancak 6. günün sonuna kadar canlılığını sürdürmüştür. Soğuk muhafaza koşullarında fermente edilen kefir ise ani bir logaritmik artış göstermemiş, en yüksek 2.87 log kob/ml seviyelerine kadar yükselmiş ve başlangıç seviyesine oranla çok belirgin bir düşüş göstermeyip, 1.98 log kob/ml seviyelerinde kalarak, 6. gün sonuna kadar canlılığını sürdürmüştür.

Anahtar sözcükler: *Kefir, Salmonella Typhimurium, Gıda patojenleri, Probiyotik, Gelişim potansiyeli*

INTRODUCTION

Kefir, which is a slightly acidic fermented dairy product, is produced from cow, ewe, goat and mare milk with the addition of kefir grains through in tandem fermentation of ethyl alcohol and lactic acid [1]. According to the Turkish Food Codex and Turkish Fermented Dairy Product Regulation, kefir is defined as fermented dairy product in which kefir

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grains or starter cultures including different strains of specific fermenting bacteria like *Lactobacillus kefiri*, *Leuconostoc*, *Lactococcus* and *Acetobacter* along with lactose-fermenting (*Kluyveromyces marxianus*) or non-fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae* and *Saccharomyces exiguous)* are used [2]. Kefir grains contain both yeasts and bacteria in their proteinaceous and carbohydrate matrix. Commonly obtained microorganisms from kefir grains are yeasts (*Kluyveromyces, Candida, Torulopsis* and *Saccharomyces* spp), different species of *Lactobacillus* genus *(L. brevis, L. casei, L. kefiri, L. acidophilus, L. plantarum, L. kefiranofaciens subsp. kefiranofaciens, L. kefiranofaciens subsp. kefirgranum, L. parakefir, L. helveticus, L. delbruecki) as well as Streptecocci (Streptecoccus salivari), Lactococcus (Lc. lactis subsp. lactis, Lc. lactis ssp. thermophilus), Leuconostoc (L. mesenteroides*), and *Acetobacter* [1,3]. These lactic acid bacteria and yeasts present in kefir grains function in fermentation of milk by producing numerous metabolites like acetic acid, lactic acid, CO_2, bacteriocin, low concentration of alcohol and aromatic substances (acetaldehyde, acetone, diacetil), which constitute the probiotic properties of kefir as a beverage [4]. These materials produced during the fermentation of kefir and other probiotic foods show inhibitory activity against growth of pathogenic bacteria like *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp. by preventing the invasion of these pathogens into the intestinal epithelium [5,6]. Kefir provides health-promoting effects and support immune system since it contains metabolites of beneficial microorganisms as well as all of the nutrients of milk [6]. Along with its health benefits, kefir is known to exhibit prophylactic and therapeutic effects in miscellaneous diseases. Kefir and kefir grains were demonstrated to have antibacterial, antifungal, antitumoral, hypocholesterolemic and immunomodulatory effects by both in vivo and in vitro studies [7].

Salmonella is a commonly pronounced microorganism included among most important pathogens responsible for food-borne poisoning. Prevalence of salmonellosis has increased in different regions of the world since mid-1980. Salmonellosis was diagnosed in 91034 people in Europe in 2002. In USA, incidence of salmonellosis was reported to be 1 million people per year, 19000 cases of which required hospitalization while 380 people died [8]. Prevalence of this disease may be associated with the acid tolerance response of *Salmonella* Typhimurium as well as its increased adaptation capability to stress factors such as heat, salt and organic acids [9-11].

Pasteurized cow milk or grayish white kefir grains are commonly used in the production of kefir. Recent public belief in homemade probiotics as being healthier products has popularized their homemade production. As a matter of course, no standardization or quality criteria are available with respect to the properties of raw material, production conditions, type of fermentation process (at room temperature or refrigerating) and shelf-life period in homemade kefir. Insufficiency in the pasteurization of milk as the raw material, overall inefficiency in performing hygienic tasks including the disinfection of equipment and utensils, filtration process of kefir yeasts and frequency of bare hand contact increase the risks for contamination. Nevertheless, even though commercially available kefir products are produced under relatively optimized industrial conditions, studies regarding the microbiological quality of the product revealed Enterococcus, Enterobacter, coliform bacteria, fecal coliforms and *E. coli* contamination [12,13].

The objective of this study was to investigate the growth potential of *Salmonella* in fermented kefir at different temperatures.

MATERIAL and METHODS

Single use natural kefir culture was used in kefir production (Danem Milk and Dairy Products Ltd.Co/Isparta). Ultra high temperature (UHT) processed, 3% fat milk (manufactured by Pınar Company) was used in the kefir production. The kefir was spiked with certificated reference culture (ATCC 14028) of *Salmonella* Typhimurium.

Preparation of Stock Culture

Lyophilized culture was initially activated according to the manufacturer's instructions and inoculated on Plate Count Agar (PCA, Merck 1.05463) to give single colonies. This stock cultures on PCA were stored at 4°C until used.

Enumeration of Salmonella in the Inoculum

To prepare a working culture solution was a single colony of *Salmonella* stock culture was transferred in to 5 ml BHIB (Brain Heart Infusion Broth, Oxoid CM1135) tube and incubated at 35°C for 18 h. The colony count in the culture solution was estimated by MacFarland densitometry (Biosan, 050102-11080341). For the enumeration of absolute *Salmonella* count, serial dilutions and plating on PCA were performed from this working culture and then incubated at 35°C for 48 h.

Preparation of Kefir Culture

For the ambient temperature fermented samples, single use kefir culture was directly added to the milk samples without prior processing. Preliminary trials showed that kefir culture was not satisfactorily activated at cold temperature; therefore it was subjected to pre-activation process in 100 ml milk (6 h at 25°C) and then added to 1 liter of milk for cold temperature fermentation.

Inoculation of the Samples with Salmonella and Incubation

The experimental design included three groups: ambient temperature fermentation group (AT group),

cold temperature fermentation group (CT group) and the control group (ATC and CTC). Milk samples with kefir grains were not inoculated with *Salmonella* consisted the control group. All of the experimental samples apart from control groups were contaminated with 100 cfu/ml *Salmonella* bacteria. All groups were subdived in to 2 parallel subgroups. After inoculation, AT group and the control samples were left at 25°C for 48 h for fermentation in an incubator (Nüve-EN 400) and then taken in to cold storage (5±1°C, Siemens). CT group and its control samples were directly left to fermentation at 5±1°C. Microbiological analyses and pH measurements were performed every 24 h during 6 days. The whole experimental procedure was repeated 3 times at different time intervals.

Microbiological Analyses

Two parallel microbiological analyses were performed for each sample and mean of these parallel results were used for data analysis. In addition, natural microbial load of the raw material (milk) was determined by enumeration of total mesophilic aerobic bacteria and detection of *Salmonella* [14,15]. Control samples were analyzed for coliform bacteria, *E. coli*, and *Salmonella* counts [15,16].

For the enumeration of *Salmonella* in spiked samples, 25 ml sample was homogenized in 225 ml 0.1% peptone water to make a 1:10 dilution. Further, serial dilutions were carried out also with 0.1% peptone water. The relevant diluted solutions were spread on selective media (XLD, Merck 1.05287 and SS, Merck 1.07667) and incubated at 35°C for 24±3 h and typical colonies were counted [15,17]. Furthermore, all samples were subjected to enrichment process to detect the presence of viable *Salmonella* below countable limit [15].

pH Measurement

pH values of the samples were measured by a pH meter (Hanna HI 2211-02) every 24 h during 6 days in two experimental (AT and CT) and in the control groups (ATC and CTC) [18].

RESULTS

In this study kefir samples were spiked with *Salmonella* and left for fermentation at two different conditions (cold and ambient temperature) and the *Salmonella* load of these samples were enumerated every 24 h for 6 days. According to the results of this study the *Salmonella* count of AT group was 2.40 log cfu/ml in the first day and decreased to 1.00 log cfu/ml at 6 day after making a peak at first 24 h and reached to 5.60 log cfu/ml. In CT group *Salmonella* count was almost steady and only increased to 2.87 log cfu/ml from 2.49 log cfu/ml at the first 24 h and it has decreased to 1.98 log cfu/ml on the last day *(Table 1, Fig. 1)*.

Microbiological analyses of raw milk did not reveal the presence of *Salmonella* and a total count of mesophilic aerobic bacteria was not determined. Likewise, *Salmonella* was not detected in the control samples (ATC and CTC) of each experimental group.

pH measurements were also carried out in *Salmonella*-

Table 1. *Growth of Salmonella Typhimurium in kefir samples of AT and CT groups (log cfu/ml)*
Tablo 1. *AT ve CT gruplarındaki kefirde Salmonella Typhimurium'un gelişimi (log kob/ml)*

Group	***Salmonella* Typhimurium Count**						
	Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
AT	2.40	5.60	4.00	2.50	1.00	1.60	1.00
CT	2.49	2.87	2.74	2.37	2.16	2.29	1.98

AT: *Kefir group fermented at ambient temperature (Trials: mean values of AT1. AT2. AT3),* ***CT:*** *Kefir group fermented at cold temperature (Trials: mean values of CT1. CT2. CT3)*

Fig 1. Changes in *Salmonella* Typhimurium quantity in AT and CT groups (log cfu/ml)

Şekil 1. AT ve CT gruplarında *Salmonella* Typhimurium'un değişimi (log kob/ml)

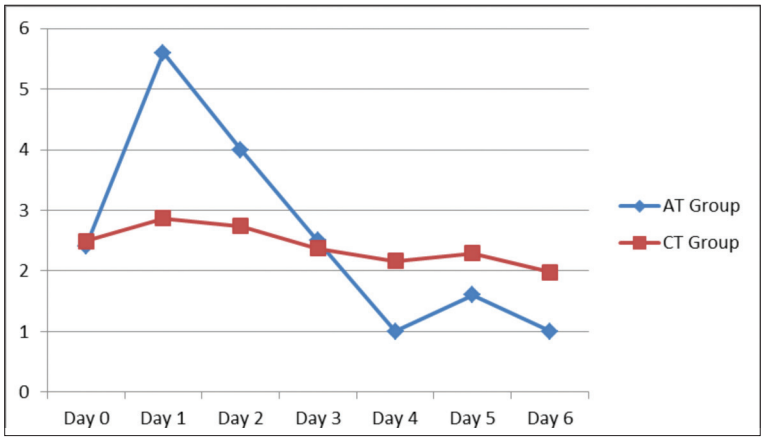

Table 2. *Changes in pH values of kefir samples in AT and CT groups*
Tablo 2. *AT ve CT gruplarındaki kefirde pH değerinin değişimi*

Group	pH						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
AT	6.78	4.48	4.24	4.28	4.30	4.28	4.31
CT	6.78	5.73	5.54	5.46	5.35	5.23	5.13

AT: *Kefir group fermented at ambient temperature (Trials: mean values of AT1. AT2. AT3),* ***CT:*** *Kefir group fermented at cold temperature (Trials: mean values of CT1. CT2. CT3)*

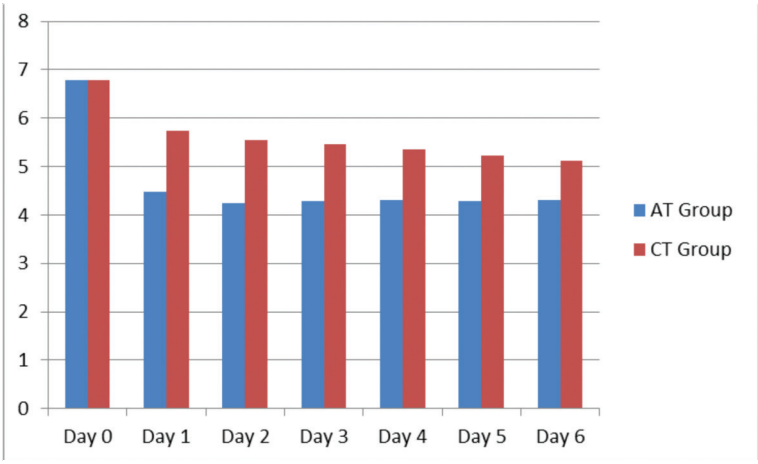

Fig 2. View of changes in pH levels in AT and CT groups

Şekil 2. AT ve CT gruplarında pH'ın değişimi

inoculated kefir at both fermentation temperatures during the whole procedure. According to the results, initial pH value of 6.78 in AT group decreased to a 4.31 level at the end of day 6 whereas that of CT group 5.13 at the end of the procedure *(Table 2, Fig. 2)*.

To monitor the unintentional contamination of samples during experimental kefir production, control samples (ATC and CTC) detected for *Salmonella* in parallel to the spiked samples. No positive detection was observed with these samples. pH measurement results of the control groups did not indicate, in comparison to the *Salmonella* contaminated groups.

DISCUSSION

According to the results of this study the growth rate of *Salmonella* was different in AT and CT groups during the first 24 h. While *Salmonella* count of AT group significantly raised from 2.40 log cfu/ml to 5.60 log cfu/ml level, CT group showed a slight change from 2.49 log cfu/ml to 2.87 log cfu/ml during the same period. Growth potential of *Salmonella* during fermentation kefir samples was previously investigated in some other studies aswell [3,6,19]. Karagozlu et al.[6] investigated the growth potential of some pathogens like *E. coli* O157:H7, *Salmonella* Typhimurium and *S. aureus* during the fermentation process of kefir at 23±1°C for 24 h. Their results showed that *Salmonella* count has increased to 4.64±0.67 log cfu/ml from 2.37±0.20 log cfu/ml at 10^2 cfu/ml *Salmonella* Typhimurium spiked samples while it has increased to 5.60±0.10 log cfu/ml from 3.52±0.07 log cfu/ml in 10^3 *Salmonella*-spiked samples at first 24 h. Our findings are mostly compatible with those of this study. Dias et al.[3] in a similar study evaluated the growth potential of *E. coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Staphylococcus aureus* and *Listeria monocytogenes* in contaminated kefir samples fermented at 20°C for 72 h, assuring a final concentration of 10^3 cfu/ml levels. According to their findings both *Salmonella* Typhimurium and *Salmonella* Enteritidis survived after 24 h. In another study by Gulmez and Guven [19] after a 24-h fermenting period (28±1°C, 20-24 h), pathogenic load of kefir regarding *E. coli* O157:H7, *L. monocytogenes* 4b and *Yersinia enterocolitica* O3 increased from 4.68±0.9 log cfu/ml to 5.32±1.1 log cfu/ml; from 4.32±0.80 log cfu/ml to 7.7±0.6 log cfu/ml and from 6.24±1.0 log cfu/ml to 7.03±1.1 log cfu/ml, respectively. In accordance with our findings and those of similar studies, when kefir is fermented at ambient temperature pathogenic microorganisms in the flora exhibits a logarithmic rise of statistical significance during the first 24-28 h [3,6,19]. In our study further fermentation after 48 h resulted with a slight decrease in *Salmonella* count in CT group, whereas it significantly dropped in AT group and at the end of the fermentation process of 6 days, AT group samples contained approximately 1.00 log cfu/ml lower *Salmonella* count than CT. Nevertheless, *Salmonella* survived at each incubation temperature till the end of determined shelf life. Likewise, Gulmez and Guven [19] demonstrated in a study that pathogenic agents in modified kefir and in pasteurized modified kefir samples survived until the 21st day of the trial with low temperature preservation. These

findings in accordance with our results proved that most pathogenic microorganisms had the capability to adapt to a changing environment like lactic acid fermentation and cold storage conditions and thus survived during the shelf-life of the product [6,8,19-21]. In contrast with our study, Dias et al.[3] did not isolate *Salmonella* in kefir samples even after 48 h of fermentation which might readily be associated with the one week enrichment process of the kefir grains they used, which eventually resulted a stronger competitive flora.

In our study, changes in pH levels were also monitored in AT and CT groups every 24 h for a 6-day period *(Table 2, Fig. 2)*. In AT group, initial pH value of 6.78 decreased to 4.48 after 24 h. This sudden change in pH level only 24 h of fermentation was consistent with the findings of several other researches [3,6,22]. Survival of *Salmonella* at such an acidic condition was considered to be associated with the acid resistance nature of the bacteria [23,24]. pH level in AT group did not show a marked change from day 2 to 6 and reached to a value of 4.3 which could be explained by the fact that growth of lactic acid bacteria started to decline in kefir matrix depending on time [22].

Initial pH value of 6.78 in CT group decreased to the levels of 5.73 in the first 24 h and 5.13 on the last day of the experiment, respectively *(Table 2, Fig. 2)*. The insignificant change in pH levels in this group could be explained by the limited activation capacity of lactic acid bacteria in kefir culture. Kefir culture contains lactic acid bacteria as well as yeasts [25]. If fermentation has occurred at low temperature, yeasts exhibit predominance in kefir flora hence alcohol fermentation develops [22].

Despite being a favorable probiotic beverage of recent years, kefir may constitute a risk for public health in case of contamination either in homemade or by industrial manufacturing. On the basis of our findings, *Salmonella* Typhimurium survived for at least six days in kefir samples fermented both at ambient and cold temperatures. Moreover, if we take into consideration that homemade kefir is usually consumed after 48-72th h of processing it is very like to expose to higher count of bacteria when fermented at ambient temperature. Therefore milk to be used as raw material should be pasteurized and utmost care should be taken in performing hygienic tasks regarding equipments and utensils, staff and the kefir culture to be used.

REFERENCES

1. Karatepe P, Yalçın H, Patır B, Aydın I: Kefir ve kefirin mikrobiyolojisi. *Elekt Mikrobiyol Derg,* 10 (1): 1-10, 2012.

2. Türk Gıda Kodeksi Fermente Süt Ürünleri Tebliği: Tebliğ No: 2009/25.

3. Dias PA, Silva DT, Tejada TS, Leal MCGM, Conceiçao RCS, Timm CD: Survival of pathogenic microorganisms in kefir. *Rev Inst Adolfo Lutz*, 71 (1): 182-186, 2012.

4. Karagözlü C, Dumanoğlu Z: Türkiyede endüstriyel kefir üretiminin arttırılması, Avrupa'da yakult pazarlaması örneği. *Gıda Teknol Derg*, 15 (11): 48-51, 2011.

5. Uymaz B: Probiyotikler ve kullanım alanları. *Pamukkale Üniv Müh Bil Derg*, 16 (1): 95-104, 2010.

6. Karagözlü N, Karagözlü C, Ergönül B: Survival characteristics of *E. coli* O157:H7 *S.* Typhimurium and *S. aureus* during kefir fermentation. *Czech J Food Sci*, 25 (4): 202-207, 2007.

7. Güven A, Güven A, Kamiloğlu NN: Kefirin lipid peroksidasyonuna etkilerinin araştırılması. *Kafkas Univ Vet Fak Derg*, 10 (2):165-169, 2004.

8. Domenech E, Belenguer AJ, Amoros JA, Ferrus MA, Escriche I: Prevalance and antimicrobial resistance of *Listeria monocytogenes* and *Salmonella* strains isolated in ready-to-eat foods in Eastern Spain. *Food Control*, 47, 120-125, 2015. DOI: 10.1016/j.foodcont.2014.06.043

9. Tosun H, Gönül ŞA: Acid adaptation protects *Salmonella* Typhimurium from environmental streses. *Turk J Biol*, 27, 31-36, 2003.

10. Leyer GJ, Johnson EA: Acid adaptation induces cross-protection against environmental stresses in *Salmonella* Typhimurium. *Appl Environ Microbiol*, 59 (6): 1842-1847, 1993.

11. Ingham SC, Su YC, Spangenberg DS: Survival of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 in cheese brines. *Int J Food Microbiol*, 61, 73-79, 2000. DOI: 10.1016/S0168-1605(00)00331-7

12. Dinç A: Kefirin bazı mikrobiyolojik ve kimyasal özelliklerinin belirlenmesi. *Yüksek Lisans Tezi,* Ankara Üniv. Sağlık Bil. Enst., 2008.

13. Angulo L, Lopez E, Lema C: Microflora present in kefir grains of the Galician region (North-West of Spain). *J Dairy Res*, 60, 263-267, 1993. DOI: 10.1017/S002202990002759X

14. Maturin L, Peeler JT: Bacteriological Analytical Manual. Chapter-3. Aerobic Plate Count. BAM, 2001. http://www.fda.gov/Food/FoodScience Research/LaboratoryMethods/ucm063346.htm; *Accessed:* 01.06.2015.

15. ISO (6579): Microbiology of food and animal feeding stuffs-Horizantal Method for the detection of *Salmonella* spp, Geneva, Switzerland: International Standards Organisation, 2005.

16. ISO (16649): Horizontal Method for the Enumeration of β- glucuronidase-positive *Escherichia coli*. Part 2-Colony-count technique at 44°C using 5-brome-4-chloro-3-indoly-beta-D-glucuronide, Geneva, Switzerland: International Standards Organisation, 2001.

17. FDA: Bacteriological Analytical Manual. Chapter-1. Food Sampling/ Preparation of SampleHomogenate.2003,http://www.fda.gov/Food/ FoodScienceResearch/LaboratoryMethods/ucm063335.htm *Accessed*: 01.06.2015

18. AOAC: Official Method of Analysis of the Association of Official Analytical Chemists. 14th ed., The Association of Official Analytical Chemists, Washington, DC. 1984.

19. Gülmez M, Güven A: Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes* 4b and *Yersinia enterocolitica* O3 in ayran and modified kefir as pre and postfermentation contaminant. *Vet Med-Czech*, 48 (5): 126-132, 2003.

20. Tosun H, Gönül ŞA: Aside adapte edilen *Salmonella* Typhimurium'un bazı asidik gıdalardaki canlılığı. *Turk J Vet Anim Sci*, 27, 1404-1407, 2003.

21. Kwarteng JO, Naneworter RO, Akabanda F: Survival of acid-adapted *Salmonella* Typhimurium in fermented millet and acidified broth at different storage temperatures. *Food Sci Qual Man*, 24, 24-31, 2014.

22. Irigoyen A, Arana I, Castiella M, Torre P, Ibanez FC: Microbiological, physicochemical and sensory characteristics of kefir during storage. *Food Chem*, 90, 613-620, 2005. DOI: 10.1016/j.foodchem.2004.04.021

23. Goverd KA, Beech FW, Hobbs RP, Shannon R: The occurence and survival of coliforms and salmonellas in apple juice and cider. *J Appl Bacteriol*, 46, 521-530, 1979. DOI: 10.1111/j.1365-2672.1979.tb00851.x

24. Roering AM, Luchansky JB, Ihnot AM, Ansay SE, Kaspar CW, Ingham SC: Comparative survival of *Salmonella* Typhimurium DT 104, *Listeria monocytogenes* and *Escherichia coli* O157:H7 in preservative-free apple cider and simulated gastric fluid. *Int J Food Microbiol*, 46 (3): 263-269, 1999. DOI: 10.1016/S0168-1605(98)00198-6

25. Güzel-Seydim ZB, Wyffels JT, Seydim AC, Greene AK: Turkish kefir and kefir grains: Microbial enumeration and electron microscobic observation. *Int J Dairy Technol*, 58, 25-29, 2005. DOI: 10.1111/j.1471-0307.2005.00177.x