

Growth of pathogens inoculated in Ayran (a Turkish drink)

Sabire Yerlikaya, İrfan Yüceer

Karamanoğlu Mehmetbey University, Engineering Faculty, Food Engineering Department, Karaman, Turkey

Abstract

Ayran is a drink made from yoghurt and contains various lactic acid bacteria, especially *Lactobacillus bulgaricus* and *Streptococcus thermophiles*. Yoghurt, ayran are valuable nutrients for our health. In this research, *Listeria monocytogenes*, *Staphylococcus aureus* growing and inhibition in ayran (Turkish drink) at 37°C for 120 minutes was investigated. At the 15th minute of incubation, *L. monocytogenes* reached 4.75 log cfu/mL, while *S. aureus* remained at 3.99 log cfu/mL. While *L. monocytogenes* concentration increases up (5.95 log cfu/mL) to the 60th minute of incubation; it was determined that the concentration of *S. aureus* increased until the 90th minute of incubation (4.49 log cfu/mL). After this period, pathogen bacterial concentrations began to decrease. It is estimated that this decrease in pathogen concentrations is caused by lactic acid bacteria and their metabolites such as bacteriocins, reuterin etc. These results demonstrated that while pathogens maintain their viability in ayran for a certain period of time, lactic acid bacteria show an inhibition effect after a while.

Keywords: *Listeria monocytogenes*, *Staphylococcus aureus*, inhibition, lactic acid bacteria

1. Introduction

Milk, yoghurt, ayran and dairy products, which are important dietary sources in human daily nutrition and sustaining life; while it is especially rich in protein and calcium, it is an important source of nutrients such as vitamins B2, B12, thiamine, niacin, magnesium and phosphorus [1]. Ayran is a drink made from yoghurt and contains various lactic acid bacteria, especially *Lactobacillus bulgaricus* and *Streptococcus thermophiles*. Yoghurt, ayran are valuable nutrients for our health. Yoghurt has many benefits for our body. It regulates immunity, has antioxidant properties thanks to fermented milk peptides, has a serum cholesterol-lowering effect, has the effect of controlling digestive system infections, has a reducing effect on lactose intolerance and has an anticarcinogenic effect [2]. The recommendation that antibiotics should not be used in order to stop the vital functions of pathogenic microorganisms that infect foods in various ways is on the agenda with the necessity of using naturally produced antimicrobial substances [3].

Listeria monocytogenes is one of the most researched and important pathogenic microorganisms for human and animal species in recent years. The most risky foods in terms of *L. monocytogenes* are foods that are ready for consumption, stored in the cold for a long time, and contain more than 2 log cfu/g of *L. monocytogenes* [4]. These foods include; products such as raw or pasteurized milk, ice cream, yoghurt and ayran etc. [5-7].

Staphylococcus species, which belong to the *Micrococcaceae* family, are Gram-positive, facultative anaerobic, non-spore-forming, non-motile and catalase-positive bacteria [8]. *Staphylococcus aureus*, which is in this family, shows high sensitivity to all applications aimed at reducing the number of microorganisms, especially heat treatment. In return, it can produce enterotoxins that cause poisoning in humans and are resistant to heat treatment [9]. *S. aureus* generally develops in foods with high protein and starch content; it is especially common in meat and dairy products (yoghurt, ayran) and foods made from them [10-11].

* Corresponding author: sabirebattal@kmu.edu.tr

In this research, it is aimed to observe growing rates of *Listeria monocytogenes* and *S. aureus* at 25°C for 120 minutes.

2. Materials and Method

2.1. Materials

Ayran were bought from local markets in Karaman, Türkiye. Pathogens, *Listeria monocytogenes* ATCC 7644 and *Staphylococcus aureus* ATCC 25923, were got from Selçuk University, Food Engineering Department in Konya. Pathogens were kept at -20°C until used.

2.2. Methods

2.2.1. Pathogens inoculation in ayran

One loopfull of *Listeria monocytogenes* and *Staphylococcus aureus* were inoculated into 10 mL of ayran sample, separately. Samples were incubated at 37°C. At the 15th, 30th, 45th, 60th, 90th and 120th minutes of incubation, 0.1 mL of the samples were taken and inoculated on Oxford Listeria Selective Agar for *Listeria monocytogenes* and BPA (Baird Parker Agar) for *Staphylococcus aureus* by the spreading plate method. The seeded petri dishes were left to incubate at 37°C for 24-48 hours. The results obtained are given as log cfu/mL.

2.2.2. Statistical analysis

The results were analyzed statistically using SPSS 22 (IBM Corp., Armonk, New York, USA) program. Sample means were compared using One Way ANOVA and were evaluated with Tukey test from Post Hoc Test.

3. Results and Discussion

As can be seen in Table 1, effects of time on *L. monocytogenes* and *S. aureus* inhibition were statistically significant ($p < 0.05$). Figure 1 shows the results of the Tukey Test for the pathogens inhibition values.

At the 15th minute of incubation, *L. monocytogenes* reached 4.75 log cfu/mL, while *S. aureus* remained at 3.99 log cfu/mL. While *L. monocytogenes* concentration increases up (5.95 log cfu/mL) to the 60th minute of incubation; it was determined that the concentration of *S. aureus* increased until the 90th minute of incubation (4.49 log cfu/mL).

The concentration of *L. monocytogenes* was found to be higher than *S. aureus* at every minute of incubation. It was observed that the concentrations of *L. monocytogenes* decreased at the 90th (5.26 log cfu/mL) and 120th minutes (4.78 log cfu/mL) of incubation. This decrease was detected at the 120th minute (4.38 log cfu/mL) of incubation for *S. aureus*. The decrease in *L. monocytogenes* concentration was not as much as in *S. aureus* concentration. Ayran is a drink made from yoghurt and contains various lactic acid bacteria, especially *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. It is estimated that this decrease in pathogen concentrations is caused by this lactic acid bacteria. Lactic acid bacteria try to inhibit pathogens in the foods by producing metabolites such as bacteriocin, reuterin and acetic acid. *L. monocytogenes* and *S. aureus* may also be affected and inhibited by these metabolites.

Table 1. Time-dependent colony amounts of pathogens

Time (min)	N	<i>L. monocytogenes</i> (log cfu/mL)	<i>S. aureus</i> (log cfu/mL)
15	2	4.75 ±0.007 ^c	3.99 ±0.007 ^b
30	2	4.79 ±0.000 ^c	4.14 ±0.028 ^b
45	2	5.09 ±0.007 ^b	4.42 ±0.050 ^a
60	2	5.95 ±0.021 ^a	4.43 ±0.057 ^a
90	2	5.26 ±0.092 ^b	4.49 ±0.042 ^a
120	2	4.78 ±0.057 ^c	4.38 ±0.106 ^a

Yıldırım et. al. [12] determined that Lactococcin BZ bacteriocin showed inhibitory activity against *L. monocytogenes* in fresh meat. In another study, it has been determined that a bacteriocin produced by *Lactococcus lactis* spp. *lactis* increases the microbial quality of meat by inoculating *Listeria innocua* in meat [13]. There are also studies indicating that lyophilized reuterine has an inhibitory effect (0.5 log for 21 days) on *L. monocytogenes* [14].

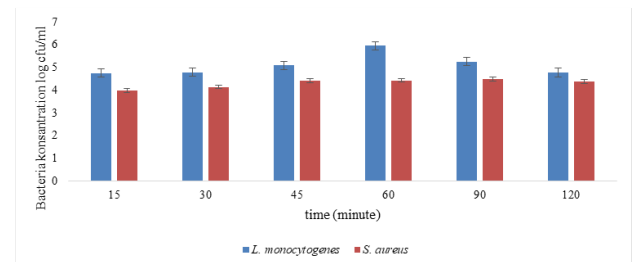


Figure 1. Inhibition of *L. monocytogenes* and *S. aureus*

From another study, the results shows different concentrations nisin (a bacteriocin) inhibited the

growth of *S. aureus*, after 4 h. Different concentrations reuterin gradually inhibited *S. aureus* ATCC 25923 and declined after 12 h [15].

4. Conclusion

Ayran is a food with high nutritional value, obtained by adding water to yoghurt. Yoghurt, which is the raw material of ayran, is a product formed as a result of the fermentation of milk with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. Since ayran is a liquid food, it is very susceptible to bacterial spoilage. As can be seen from our study, when contaminated with *L. monocytogenes* and *S. aureus* as pathogenic bacteria, pathogen concentrations increase to a serious level in a very short time. Although they are inhibited up to a certain concentration by various lactic acid bacterial metabolites in the composition of ayran, the use of different natural preservatives is also needed.

The results of this study show, when ayran, which is a dairy product, is contaminated with *L. monocytogenes* and *S. aureus*, they can multiply rapidly and shortens shelf life. It has also been determined that *L. monocytogenes* is a bacteria that reproduces faster than *S. aureus*. It can also be thought that *L. monocytogenes* transitions from the lag phase to the log phase of bacterial development faster than *S. aureus*.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

Disclosure statement. No potential conflict of interest was reported by the authors.

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