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Türk Hijyen ve Deneysel Biyoloji Dergisi

Effects of beverages taken with meal on some foodborne pathogens in simulated gastric fluid

Yemek yanında tüketilen içeceklerin simüle mide ortamında gıda patojenleri üzerine etkisi

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ABSTRACT

Objective: Although it is thought that human stomach acidity would inhibit microorganisms, when food poisoning cases are considered, it is seen that especially during consumption of meat and meat products, the pH in the stomach rises and the fat layer creates a protective effect on bacteria, allowing microorganisms to transfer to intestine, and subsequently cause foodborne illnesses. The aim of this study was to determine whether beverage intake with meals influences why some individuals get sick, while others do not in food poisoning outbreak cases.

Methods: The effect of coke, ayran and shalgam, which are popularly consumed beverages along with meals in Turkey, was examined on the survival of *Salmonella enteritidis*, *Listeria monocytogenes*, *Escherichia coli* O157: H7 and cocktail of these pathogens in simulated gastric fluid (SGF). Doner, a worldwide popular fast-food product, was used as the food sample.

Results: Shalgam was found to be noteworthy that provided the greatest reduction in pathogen count.

ÖZET

Amaç: İnsan mide asitliğinin mikroorganizmaları inhibe edeceği düşünülse de gıda zehirlenmesi vakalarına bakıldığında, özellikle et ve et ürünleri tüketimi sırasında midede pH'nin yükselmesi ve yağ tabakasının bakterilere koruyucu etki oluşturmasıyla birlikte mikroorganizmaların bağırsağa geçişine, dolayısıyla gıda zehirlenmelerine olanak sağladığı görülmektedir. Bu çalışmanın amacı, toplu gıda zehirlenmelerinde neden bazı bireylerin hastalandığını, bazılarının ise etkilenmediğini ve gıda yanında tüketilen içeceğin bu durumu etkileyip etkilemediğini belirlemektir.

Yöntem: Bu çalışmada, gıda örneği olarak dünyaca tüketimi yaygın olup hijyen seviyesi düşük ve birçok gıda zehirlenmesi vakasında adı geçen döner kullanılmıştır. Döner yanında tüketilen yaygın içeceklerden ayran, kola ve şalgam suyunun çeşitli döner ve içecek miktarlarında dönerde bulunması muhtemel patojen bakterilerden *Salmonella enteritidis*, *Listeria monocytogenes*, *Escherichia coli* O157: H7 ve patojen karışımı sayısı üzerine, simüle edilmiş mide ortamındaki etkisine bakılmıştır.

Bulgular: Yapılan bu çalışma ile patojen sayısında

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It was observed that in vitro consumption of one serving (300 mL) of shalgam, coke, and ayran along with contaminated doner, reduced the pathogenic bacteria count in SGF up to 2.6, 2.25 and 1.6 log CFU/g, respectively. The results showed that consuming those beverages alongside one portion (100 g) of low contaminated (~101 CFU/g) doner might eliminate the possibility of a healthy person having foodborne illness due to aforementioned bacteria.

Conclusion: It can be concluded that, in food poisoning cases, not only the personal immune system, but also the type and amount of beverage consumed along with food, as well as the amount of food intake are effective.

Key Words: Simulated gastric fluid, beverage, *Listeria monocytogenes*, *Salmonella enteritidis*, *Escherichia coli* O157: H7

en fazla azalmayı sağlayan içeceğin şalgam suyu olduğu dikkati çekmektedir. Bir porsiyon (300 mL) şalgam, kola ve ayran'ın kontamine dönerle birlikte in vitro tüketiminin simüle mide ortamındaki patojen bakteri sayısını sırasıyla 2,6; 2,25 ve 1,6 log CFU/g'ye kadar düşürdüğü gözlendi. Sonuçlar, bu içeceklerin bir porsiyon (100 g) düşük kontamine (~101 CFU/g) dönerle birlikte tüketilmesinin, sağlıklı bir kişinin yukarıda belirtilen bakteriler nedeniyle gıda kaynaklı hastalığa yakalanma olasılığını ortadan kaldırabileceğini göstermiştir.

Sonuç: Sonuç olarak, gıda zehirlenmeleri vakalarında kişisel bağışıklık sisteminin yanısıra, tüketilen gıdanın miktarı ve yanında tüketilen içeceğin cinsi ve miktarının da etkili olduğu kanısına varılmıştır.

Anahtar Kelimeler: Simüle mide ortamı, alkolsüz içecek, *Listeria monocytogenes*, *Salmonella enteritidis*, *Escherichia coli* O157: H7

INTRODUCTION

Foodborne pathogenic bacteria cause foodborne diseases and deaths every year, as well as economic losses. Although stomach acidity, which has a pH of 1.5-2 in a healthy person, is expected to inhibit microorganisms completely, pathogens can get rid of this acidity, reach the intestine, increase in number, and cause illness. The increase of pH in the stomach that occurs during food poisoning, especially during the consumption of meat products, allows this transfer of microorganisms to the intestine. Moreover, it has been observed that the fat layer of food often covers the pathogen like a blanket, protecting it from the negative effects of stomach acidity. In cases of food poisoning outbreaks, it has been observed that some individuals get sick, while others do not. It is known that one's personal immune system, endurance and diet are effective in this difference. It is also clear that the amount of food consumed is proportional to the number of pathogens taken into the body (1-3).

Doner kebab is a meat-based food originating from the Middle East, which is mostly preferred by young people as a fast-food all over the world. It is a product with a very high rate of open-air sale. Doner is known as gyro, donair, donar kebab, souvlaki, chawarma or shawarma in various parts of the world (4-6). In the past years, outbreaks caused by doner kebab have been reported in different regions of the world (7-9). In studies conducted in various countries, it has been found that doner kebabs have low hygiene levels and are contaminated with a group of microorganisms, including pathogens such as *Escherichia coli* O157:H7, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Clostridium perfringens* (4, 9-11).

According to studies, ayran is the most common beverage, followed by coke, in Turkey, where doner is consumed the most (12, 13). Shalgam is another beverage which is preferred especially along with

meat meals in Turkey. Ayran is a fermented milk-based beverage traditionally produced by mixing yogurt with water and salt. Shalgam (fermented turnip juice) is another traditional beverage unique to Turkey. Shalgam root, black carrot, red beet, bulghur flour, water, yeast and salt are used in the production of shalgam, which has a strong flavor resulting from lactic acid fermentation (14, 15). Ayran has a pH between 4.1 and 4.6, whereas the pH of shalgam varies between 3.2 and 3.7 (16-18). Another worldwide popular beverage, coke, has higher acidity than either ayran or shalgam with the pH between 2.3 and 2.5 (19, 20).

Pathogen survival studies in simulated gastric fluid (SGF) have been carried out in the presence of either only food or the beverage. The only beverage studied in SGF along with food was wine and it was determined that wine has a high inhibitory effect on the studied pathogens (1, 21-26). There were no studies in literature researching the effect of soft drink consumption along with meat which causes the actual increase in gastric pH.

Considering the positive results obtained in the studies conducted with wine in SGF, it was predicted that fermented products such as ayran, shalgam, and coke, with higher acidity, would also have the same effect in inhibiting pathogens during the consumption of a meat-based meal. The aim of this study was examining the effect of coke, ayran and shalgam, which are popularly consumed along with doner on the survival of some pathogens likely to be found in doner such as *Salmonella enteritidis*, *L. monocytogenes*, *E. coli* O157:H7 in SGF. The minimum amount of beverages providing the inhibition of all microorganisms were also investigated within the scope of this study.

MATERIAL and METHOD

Materials

Doner, coke, ayran and shalgam samples were obtained from supermarkets (Çorum region, Turkey) in sealed packages within the expiry date. Preservative food additives in the samples were avoided. Doner samples were leaf doner, made of veal and lamb mixture. All the materials were stored at 4°C until used. Each beverage was stored within the expiry date and used freshly opened on the day of analysis. Doner samples were autoclaved at 121°C for 15 min, while beverage samples were used without any sterilization process except for Minimum Bactericidal Concentration (MBC) analysis. To prevent lactic acid bacteria growth during MBC analysis, ayran and shalgam samples were filter sterilized.

Tryptic Soy Broth (TSB) (Merck 1.05459), Tryptic Soy Agar (TSA) (Merck 1.05458), anhydrous D-glucose (Merck CAS 50-99-7), 37 % HCl for analysis (Merck 100317), $CaCl_2$ (Merck 102378), KCl (Merck CAS 7447-40-7) and extra pure NaCl (Merck CAS 7647-14-5) for analysis were obtained from Merck, Darmstadt, Germany. Mueller Hinton Agar (MHA) (CAS 335-64-8), proteose-peptone (91079-38-8), KH2PO4 (CAS 7778-77-0), bile extract porcine (CAS 8008-63-7) and pepsin porcine (CAS 9001-75-6) gastric mucosa powder were supplied from Sigma-Aldrich, St. Louis, MO, USA. Lysozyme from hen egg white (10837059001) was obtained from Roche, Mannheim, Germany. Membrane filter with 0.45 µm pore was supplied from M&N Nagel, Düren, Germany.

Bacterial strains

L. monocytogenes ATCC 19115, *S. enteritidis* ATCC 13076 and *E. coli* O157:H7 ATCC 43889 were obtained from Ankara University Faculty of Engineering Food Engineering Department Food Microbiology Laboratory. One day before each doner adaptation, bacteria were activated by incubating in TSB for 24 h at 37°C. Each strain was inoculated (as described below) onto doner samples individually and mixed for pathogen cocktail.

Preliminary tests were done in order to show if there is any significant difference in adapted and non-adapted inoculums of doner samples. For the non-adapted samples, the doner samples were inoculated

by the active culture on the day of analysis. On the other hand, for adapted doner samples, doner samples were inoculated one day before each analysis and kept in 4°C overnight. Preliminary tests showed that there is remarkable decrease (≥3 log CFU/g) in bacteria count when doner is not adapted thus, it was determined that adaptation is necessary.

Simulated Gastric Fluid

The model stomach system was developed in vitro, using SGF and was prepared according to Yuk and Schneider, 2006 (24) except that bile extract porcine was used instead of ox bile. The conditions were determined based upon human physiological conditions of gastric fluid volume, pH, and transit time. Considering that red meat is one of the most difficult foods to digest, digestion time and shaking speed were kept at a maximum of 200 rpm in a shaking water bath for 4 h at 37°C. SGF was prepared fresh daily by dissolving 8.3 g proteose-peptone, 3.5 g D-glucose, 2.05 g NaCl, 0.6 g, KH_2PO_4, 0.11 g CaCl2, 0.37 g KCl in 1 L of distilled water. The mixture was autoclaved at 121°C for 15 min; and 0.05 g bile extract porcine, 0.1 g lysozyme and 13.3 mg pepsin were added by filter sterilizing. The pH was adjusted to 1.5 with 25 % HCl.

Sample preparation and fraction plan

Prior to use, pH values of beverages were measured. After sample preparation, pH values of non-contaminated sample filtrates were also determined in triplicate.

The dilutions were prepared for three different inoculums: high, moderate and low inoculum. The activated cultures in TSB has been found to contain 5.8 and 7.1 x 10^8 CFU/mL, thus the high contaminated samples were inoculated by 1 mL of direct active culture onto doner samples. The final inoculum sizes were approximately 10^7 CFU/g for high inoculum. In order to prepare moderate and low inoculum, the active cultures were diluted (via %0.85 NaCl) for 3 and 6 times respectively so that having the final inoculum sizes would be approximately 104 and 10^1 CFU/g, respectively. The inoculation was done by 1 mL of the proper dilutions added onto 80 g doner + 20 mL of sterile physiological saline solution (PSS) and homogenized by stomacher paddle blender (Interscience BagMixer 400P).

For the pathogen cocktail preparation, 1 mL of each of the active cultures of the afore-mentioned three pathogen strains were inoculated onto doner samples, having the final inoculum sizes of 6.73 ± 0.15 log CFU/g (referred to as high inoculum), 4.27 ± 0.22 log CFU/g (referred to as moderate inoculum) and 1.1 ± 0.11 log CFU/g (referred to as low inoculum). The changes in the number of the pathogen cocktail were followed in terms of total mesophilic aerobic bacteria count (TMAB). Since the sensitivity in low inoculum was 1 CFU/g; thus, the precision for low inoculum could only be provided by MBC analysis. Doner adaptation of the bacterial strains was made by storing the contaminated doner samples at 4oC for 24 h.

The fraction plan is summarized in Figure 1 and the sampling plan is depicted in Figure 2. Traditionally, doner kebab is consumed mostly in pita/bread as 100 g doner+300 g pita or served in a plate as 200 g doner with one serving (300 mL) of beverage consumed alongside. Hence, the fraction plan (Fig. 1) was made into 2 plans: 1- Good scenario (GS): Small amount of doner + large amount of beverage consumption; 2- Bad scenario (BS): Large amount of doner + small amount of beverage consumption. The gastric fluid volume changes according to the solid food amount inside.

For each different occasion, 10 stomacher bags were prepared (Fig. 2): Two bags as control (without either beverage or SGF); 2 bags as SGF control (without beverage) and 6 bags with beverages (2 different scenarios for each sample). When proportioned, the GS samples were composed of 6.25 g contaminated doner, 25 mL SGF and 18.5 mL of either coke, shalgam or ayran. The GS control sample was without beverage, but with 43.5 mL of ; SGF control was without beverage, but with 18.5 mL of PSS. The BS

samples were composed of 25 g contaminated doner, 25 mL SGF and 12.5 mL of beverage. The BS control sample was without beverage, but with 37.5 mL of PSS; SGF control was without beverage, but with 12.5 mL of PSS.

Bags were sampled after previously stated water bath incubation and enumeration of cell count was made by spread plate method on TSA and incubated overnight at 37°C.

Minimum bactericidal concentration (MBC)

The minimum amount of beverages providing the inhibition of all bacteria were also investigated within the scope of this study. The pathogen cocktail of 1-3 log CFU/g was inoculated onto doner samples and the fraction plan was carried out as previously described (Fig. 1). The beverage amounts were proportioned for every 0.5 portion, from 0.5 to 5 portions of each beverage (300 mL for each beverage portion). After the simulated digestion process, 0.1 mL from each sample was inoculated onto 5 mL of TSB. Any turbidity in TSB after incubation at 37°C for 24 h was evaluated as positive in bacterial growth. Streaking was performed from the negative tubes onto MHA. The plates were incubated at 37°C for 24 h. The beverage amount that no bacterial growth observed was recorded as MBC. The experiments were performed in triplicate.

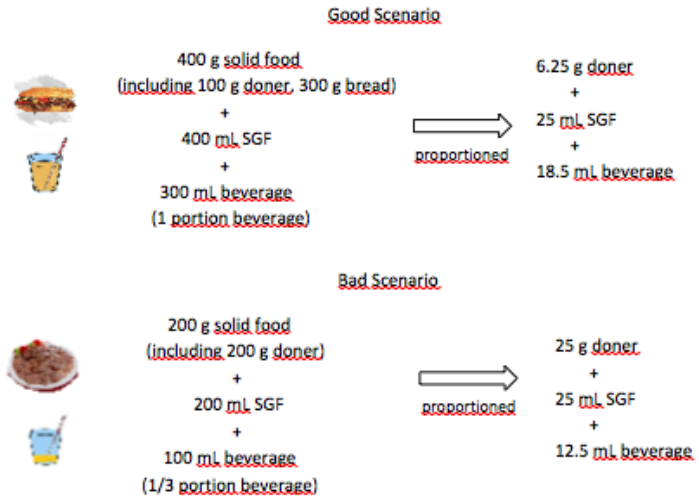

Figure 1. Fraction plan. SGF: Simulated gastric fluid

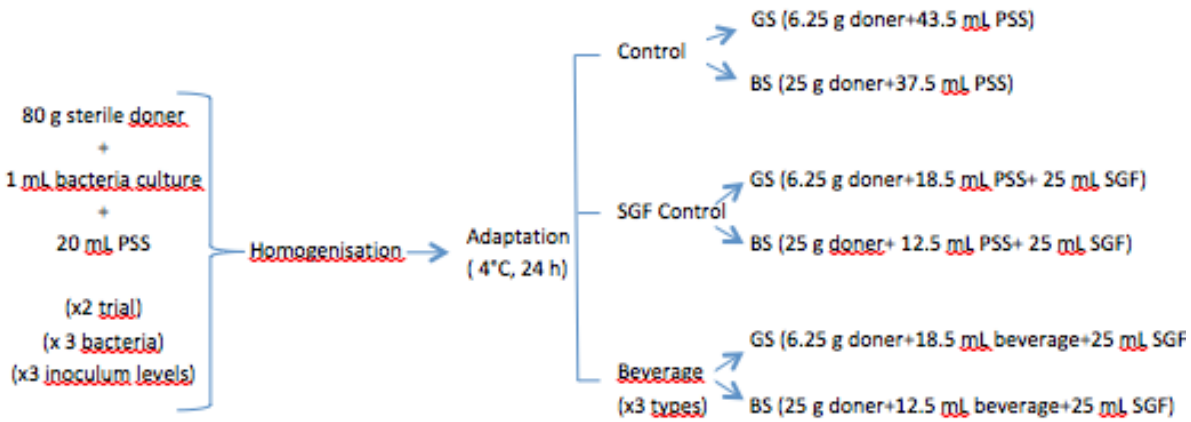

Figure 2. Sampling plan. SGF: Simulated gastric fluid. PSS: Physiological saline solution

Statistical analysis

Microbiological experiments were done in duplicate whereas pH was measured in triplicate, each of which was performed on different occasions. Variance analysis was performed using the SAS 9.0 program. In cases where differences were found, the Least Significant Difference (LSD) multiple comparison test was used to determine whether the difference was significant ($P<0.05$). The significant statistical difference in the microbiological value means that the applied procedure reduces the microbial load. The graphs were composed using MS Office Excel 2010 Program.

RESULTS

pH shift evaluation

Coke, ayran and shalgam purchased from the market had a pH of 2.30 ± 0.16, 4.08 ± 0.11 and 3.09 ± 0.15, respectively. The SGF, which was adjusted to a pH of 1.58 ± 0.21, reached up to a pH of 4.81 ± 0.39, depending on the amount of doner without beverage. In the presence of a large amount of coke, ayran, and shalgam consumption with a small amount of doner (GS), the pH decreased to 3.10 ± 0.26, 3.44 ± 0.06 and 3.27 ± 0.43, respectively. In the BS, pH decreased to 4.39 ± 0.19, 4.67 ± 0.10 and 4.59 ± 0.21 with coke, ayran, and shalgam, respectively.

Inactivation of pathogens in SGF during beverage consumption along with doner

The changes in the number of the pathogen cocktail in terms of TMAB in the model stomach system with beverages consumed along with doner are given in Table 1. In the GS-high inoculum, the number of pathogen cocktail which was 6.73 ± 0.15 log CFU/g, decreased significantly with each of SGF, coke, ayran, and shalgam ($P<0.05$). The number of pathogen cocktail decreased approximately 1 log CFU/g when they entered the stomach with doner, and there was no significant change in their number with the addition of coke and ayran after entering in SGF ($P> 0.05$). After entering the model stomach, the only difference in the number of pathogens was realized in shalgam, statistically. Shalgam provided approximately 2.5 log CFU/g reduction in pathogen count compared to the control sample, and 1.5 log CFU/g reduction compared to SGF control which did not contain beverage. GS-moderate inoculum showed similar results with high inoculum. All applications decreased the pathogen count compared

Table 1. The changes in the number of the pathogen cocktail in terms of Total Aerobic Mesophilic Bacteria Count (TAMB) in model stomach system with beverages along with doner

	The number of aerobic plate counts (log_{10} CFU/g)					
	GS			BS		
	High Inoc.	Mod Inoc.	Low Inoc.	High Inoc.	Mod Inoc.	Low Inoc.
Control	6.73 ± 0.15^{a}	4.27 ± 0.22^{a}	1.10 ± 0.11^{a}	7.34 ± 0.16^{a}	4.65 ± 0.15^{a}	1.74 ± 0.23^{a}
SGFcontrol	5.78 ± 0.35^{b}	3.62 ± 0.33^{ab}	0.33 ± 0.16^{b}	7.05 ± 0.2^{a}	4.46 ± 0.19^{a}	1.90 ± 0.20^{a}
Coke	5.56 ± 0.37^{b}	3.13 ± 0.7^{bc}	0.26 ± 0.19^{b}	7.18 ± 0.15^{a}	4.44 ± 0.20^{a}	1.79 ± 0.18^{a}
Ayran	5.43 ± 0.35^{b}	3.02 ± 0.86^{bc}	0.21 ± 0.21^{b}	6.99 ± 0.19^{a}	4.33 ± 0.20^{a}	1.11 ± 0.46^{a}
Shalgam	4.24 ± 0.29^{c}	2.18 ± 0.54^{c}	0.19 ± 0.19^{b}	6.91 ± 0.16^{a}	4.33 ± 0.11^{a}	1.76 ± 0.21^{a}

Data represents mean ± SD (n=2). Means followed by different lower-case letter superscript in a column differ significantly ($P< 0.05$). GS: Good scenario (Small amount of doner with large amount of beverage); BS: Bad scenario (Large amount of doner with small amount of beverage). Inoc.: Inoculum.

to the control, yet the only statistical difference was observed with shalgam compared to SGF control (P <0.05). In the moderate inoculum, a 2 log CFU/g reduction in pathogen count was obtained by Shalgam compared to the control sample and 1.5 log CFU/g compared to the SGF control. In the GS-low inoculation, although all applications made a statistically significant difference (P <0.05) compared to the control, no beverage showed a difference (P> 0.05) compared to SGF control. It was observed that the reason for this was that the sensitivity of analysis was 1 CFU/g in low inoculum, and results with <1 CFU/ g were considered zero. Hence, low inoculum results were evaluated by MBC analysis results.

Unlike the GS, in all of the high, moderate and low inoculations of the BS, none of the applications provided a significant reduction in the number of pathogen cocktail (P> 0.05). The maximum reduction was achieved by shalgam with 0.4 log CFU/g which ends up with no statistically significant difference in the number of pathogens (P> 0.05).

The effects of beverage consumption along with high contaminated doner on *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 are individually shown in Figure 3. In the BS, where the beverage amount was less, neither SGF control, nor the beverage intake along with doner affected any of the pathogen count significantly (P> 0.05). In the GS, where the amount of drink is approximately one portion (300 mL), shalgam was the beverage that provided the highest reduction, among the others, for all bacterial species.

In the GS, high inoculum; entering the model stomach (SGF) reduced the number of *L. monocytogenes* and *E. coli* O157:H7 significantly (P< 0.05) yet did not affect *S. enteritidis* count (P> 0.05). Each beverage inhibited *L. monocytogenes* significantly (P< 0.05). Coke and shalgam showed the same inhibition effect with 2.25 and 2.6 log CFU/g reduction, respectively, for *L. monocytogenes*; while ayran reduced the count to 1.28 log CFU/g, yet increased the number slightly compared to SGF control. Although *E. coli* O157:H7 count decreased compared to SGF control, the only beverage that inhibited *E. coli* O157:H7 significantly after entering the model stomach was shalgam (P< 0.05). The decrease in *E. coli* O157:H7 number were 0.83, 0.72, and 2.03 log CFU/g in the presence of coke, ayran, and shalgam, respectively, which were all less than other bacteria count decreases. *S. enteritidis* was not affected significantly by coke or ayran; only shalgam showed an inhibition effect. The decreases in *S. enteritidis* numbers were 0.8, 1.6, and 2.4 log CFU/g in the presence of one portion of coke, ayran and shalgam, respectively.

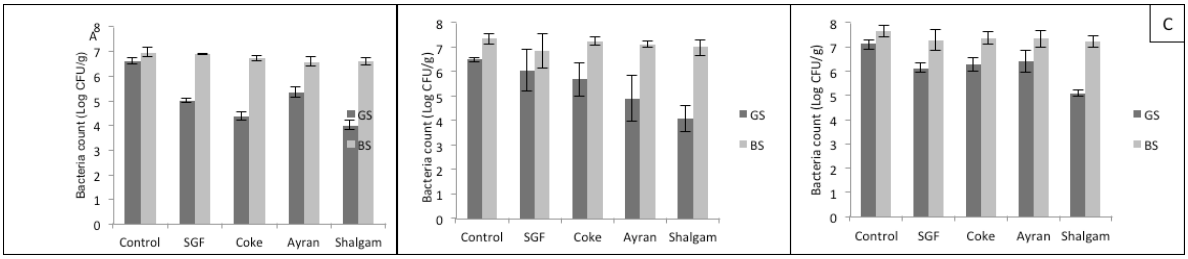

Figure 3. The effect of beverages along with highly contaminated (~10^7 CFU/g) doner on *Listeria monocytogenes* (A), *Salmonella enteritidis* (B), and *E. coli* O157:H7 (C) count in SGF. GS: Good scenario (Small amount of doner with large amount of beverage consumption) BS: Bad scenario (Large amount of doner with small amount of beverage consumption). Data bars represent the mean and the error bars represent the standard deviation of duplicate for each bacterial strain. The bacteria counts indicated by different letters within the GS or BS bars are significantly different (P> 0.05)

Figure 4 shows the effect of beverage consumption along with moderately contaminated doner on *L. monocytogenes*, *S. enteritidis*, and *E. coli* O157:H7, respectively. In the BS, moderate inoculums showed similar results as in high inoculums. When doner consumption is high and the beverage consumption is low, pathogen counts were not affected significantly (P> 0.05). In the GS, shalgam was the beverage that provided the highest reduction, among the others, for *L. monocytogenes* and *E. coli* O157:H7 (P< 0.05). Unlike other species, *S. enteritidis* was not affected significantly (P> 0.05) by any beverage intake, including shalgam.

In the GS, moderate inoculum; entering the model stomach (SGF) reduced the number of *L. monocytogenes* and *E. coli* O157:H7 significantly (P< 0.05) yet did not affect *S. enteritidis* count (P> 0.05); all were similar to high inoculum results. Similarly, as in high inoculum, coke and shalgam showed the same inhibition effect on *L. monocytogenes*. The decrease in count was 1.17 log CFU/g for coke and 1.44 log CFU/g for shalgam. Ayran decreased *L. monocytogenes* count to 0.55 log CFU/g showing no more different effect than SGF (P> 0.05). Even though the number of *S. enteritidis* showed the greatest decrease of all species by each beverage intake; none of SGF, coke, ayran or shalgam made a significant difference in number, statistically (P> 0.05). Coke, ayran and shalgam decreased the number of *S. enteritidis* to 1.11, 1.5 and 1.8 log CFU/g, respectively, during doner consumption. The decrease in *E. coli* O157:H7 numbers were 0.49, 0.69, and 1.3 log CFU/g in the presence of coke, ayran and shalgam, respectively, when moderately contaminated.

The effects of beverages on the difference between pathogen species were evaluated in Table 2. Since no beverage provided significant inactivation at any inoculum level in the number of bacteria in the BS, the inactivation percent assessments were made only for the good scenario. When pathogens entered the model stomach at high rates with doner, *L. monocytogenes* was found to be the bacterium that was the most sensitive when no beverage was consumed. The most resistant bacterium under these conditions was found to be *S. enteritidis*. *E. coli* O157: H7 was inactivated at the highest level if the moderate level of bacteria entered the stomach without any beverage. Among the beverages, shalgam provided the most inactivation with an average of 99.5 %. *L. monocytogenes* was inactivated the most (99.75 %) among all pathogen species studied. Shalgam showed the same inactivation effect on all bacteria in high inoculum (P> 0.05), moreover Shalgam significantly inactivated all pathogenic bacteria at both inoculum levels. In high inoculum, the most resistant pathogen to Ayran was *E. coli* O157: H7. *S. enteritidis* was found to be as sensitive as *L. monocytogenes* is to Ayran. On the other hand, while the most sensitive species to Coke in high inoculum

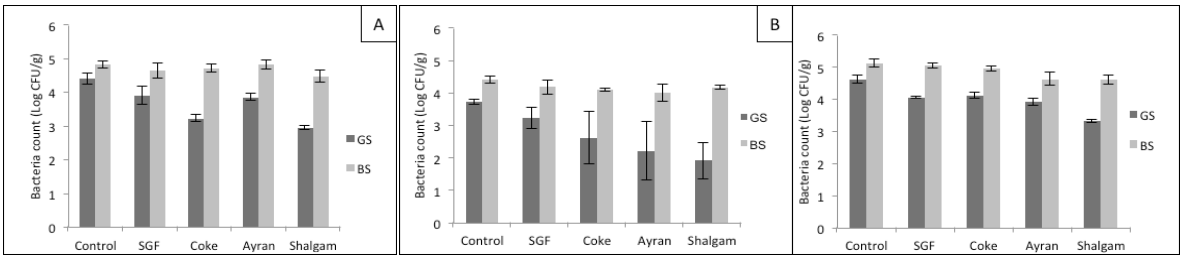

Figure 4. The effect of beverages along with moderately contaminated (~10^4 CFU/g) doner on *Listeria monocytogenes* (A), *Salmonella Enteritidis* (B), and *E. coli* O157:H7 (C) count in SGF. GS: Good scenario (Small amount of doner with large amount of beverage consumption) BS: Bad scenario (Large amount of doner with small amount of beverage consumption). Data bars represent the mean and the error bars represent the standard deviation of duplicate for each bacterial strain. The bacteria counts indicated by different letters within the GS or BS bars are significantly different (P> 0.05)

was *L. monocytogenes*, the same level of resistance was observed in *E. coli* O157: H7 and *S. enteritidis*.

Minimum Bactericidal Concentration

Low inoculum results were evaluated by MBC analysis. The results of MBC analysis were given in Figure 5. The beverage portions that inhibit all pathogens were determined for ~1 and 2 log CFU/g including doner samples. One portion of coke and shalgam and 1.5 portion of ayran inactivated all the microorganisms in SGF when the doner samples were contaminated with ~10^1 CFU/g. When doner samples were contaminated with ~10^2 CFU/g, MBC was determined as 3.5, 4.5, and 3 portions for coke, ayran, and shalgam, respectively. However, when doner was contaminated with ~3 log CFU/g of pathogens, turbidity was observed in all the samples after the SGF, thus no MBC values were obtained.

Table 2. Inactivation rates of pathogens during gastric conditions in the presence of beverages

		Inactivation percentage (%)		
		S. enteritidis	*L. monocytogenes*	*E. coli* O157:H7
High Inoculum	SGF	63.69 ± 1.11^{c}	97.49 ± 0.12^{a}	89.77 ± 0.07^{b}
	Coke	84.15 ± 0.12^{b}	99.44 ± 0.15^{a}	85.21 ± 0.11^{b}
	Ayran	97.49 ± 1.55^{a}	94.75 ± 0.12^{a}	80.95 ± 0.13^{b}
	Shalgam	99.60 ± 0.14^{a}	99.75 ± 0.20^{a}	99.07 ± 0.09^{a}
Moderate Inoculum	SGF	68.26 ± 0.21^{b}	67.64 ± 0.10^{b}	72.46 ± 0.09^{a}
	Coke	92.18 ± 0.13^{a}	91.49 ± 0.11^{a}	67.64 ± 0.22^{b}
	Ayran	96.88 ± 0.78^{a}	71.82 ± 0.22^{c}	79.58 ± 0.53^{b}
	Shalgam	98.42 ± 0.64^{a}	96.37 ± 0.73^{a}	94.99 ± 1.78^{a}

Data represents mean ± SD (n=2). Means followed by different lower-case letter superscript in a row differ significantly ($P< 0.05$). SGF: Simulated Gastric Fluid.

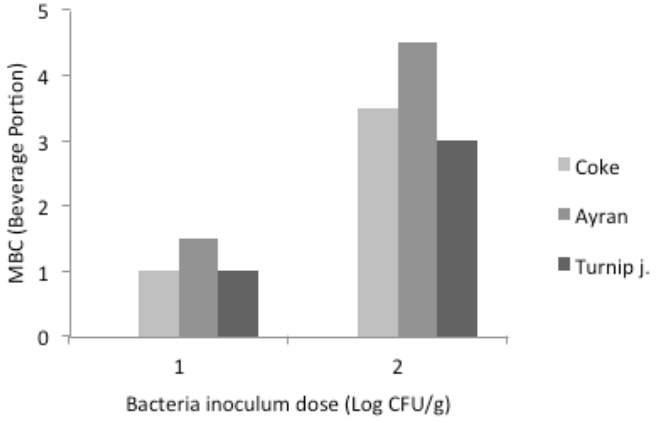

Figure 5. MBC values of beverage portions consumed along with doner contaminated by pathogen cocktail (one portion beverage=300 mL).

DISCUSSION

In this study, the effect of beverages (coke, ayran and shalgam) in simulated gastric conditions on pathogenic bacteria such as *S. enteritidis*, *L. monocytogenes* and *E. coli* O157:H7 that can possibly contaminate doner, a widespread, popular fast-food item which has a low hygiene level, was studied. No studies were found in the literature that investigated the effect of a soft-drink consumed along with meat in gastric conditions on foodborne pathogens.

This study determined that shalgam is the most noteworthy of all the beverages investigated as it provided the greatest reduction in the number of pathogens. However, coke and ayran were found to have similar effects on some bacterial species (coke for *L. monocytogenes* and ayran for *S. enteritidis*). It was observed that the inhibition effect of shalgam did not differ among pathogens, and it was determined that it is the most likely beverage to prevent food poisoning by providing a reduction of up to 2.6 log CFU/g when one portion (300 mL) is consumed by a healthy person. When approximately one can of coke and ayran was consumed along with pathogen contaminated doner, the number of pathogens decreased up to 2.25 and 1.6 log CFU/g, respectively.

The pH of SGF itself increased when doner was consumed and after beverage intake, pH got lower again. The lowest pH was achieved with high coke intake, but coke was not the beverage which achieves the most microbial inhibition. The most microbial inhibition was observed in high shalgam consumption, which has an average pH value. The acidity of coke derives from carbonic and phosphoric acids which decrease the pH by carbonating during the production (27, 28). It was found in this study that coke caused a significant decrease in the number of *L. monocytogenes* owing to its high acidity. Unlike coke, the acidity of ayran and shalgam is derived from lactic acid, which is an organic acid. Ayran and shalgam decreased the bacteria count with the effect of antagonistic activities of the lactic acid bacteria against the pathogens, as well as the by-products produced during the fermentation in addition to pH. Moreover, some certain components derived from the raw materials of ayran and shalgam also have antimicrobial effects on pathogens.

Starter culture used in ayran production, *Lactobacillus delbrueckii* spp. *bulgaricus* and some strains of *Streptococcus thermophillus*, has been reported to have an inhibitory effect on pathogens (14, 29). However, it was also shown by further studies that pathogens can survive during the production stages (30, 31). Although it was determined that ayran has a slight inhibition effect on some pathogens in the SGF in this study, both coke and shalgam were found to be more effective than ayran. An explanation for this is that ayran has a higher pH value than the other beverages. Ayran did not reduce the number of *L. monocytogenes*, S. enteritis, and *E. coli* O157: H7 in doner samples significantly with high and moderate levels of pathogens. If doner is contaminated with a low number of pathogens, then ayran has an inhibitory effect, eliminating the risk of food poisoning if a 1.5 portion (450 mL) is consumed.

It is known that *Lactobacillus plantarum*, which is used as a starter culture in shalgam production, has an antagonistic effect against pathogenic microorganisms (15). It has been proven by studies that red beet and black carrot used in the production of shalgam have antimicrobial effects on many pathogenic microorganisms, mostly on *L. monocytogenes* (32, 33). This study found that the inhibition effect of shalgam was the same on *L. monocytogenes*, *S. enteritidis*, and *E. coli* O157:H7, decreasing the numbers significantly.

In the BS at all inoculum levels, no significant change in the number of pathogen cocktail and any individual pathogen species was observed. This demonstrates that the portion amount consumed is important in food poisoning cases. Obviously, the larger the portion size is, the higher the amount of pathogen entering the stomach and, therefore, into the intestine. In addition,

proportioning the amount of beverage consumed with the food portion size is important in order to decrease the risk of having foodborne illnesses.

Infective dose is the ingestion of pathogenic microorganisms into the body in an amount that makes people sick. The infective dose depends on the strains used, the age and physical condition of the individuals; therefore, it might vary in a wide range. In addition, since many of the volunteer studies were conducted by feeding the organisms into a non-food matrix after neutralizing gastric acidity, the results obtained may not reflect the true dose response. Nevertheless, the common infective doses for the studied pathogens can be indicated as follows: 1-10 CFU/g for *E. coli* O157:H7; 10^2- 10^5 CFU/g for Salmonella spp.; and 10^3-10^5 CFU/g for *L. monocytogenes* (34-37). In cases where there are 10^6-10^7 CFU/g-mL microorganisms in a food, food spoilage becomes noticeable (2). Therefore, in light of this information and the results obtained from this study, in case doner samples are highly contaminated, doner will not be consumed or sold anyway. On the other hand, if doner kebab samples contain low levels of pathogenic microorganisms, consuming small portions of doner and having any of these three beverages truly proportioned, can prevent foodborne illnesses. The most dangerous situation can occur in moderately contaminated doner samples. In this case, it has been observed that one portion of Shalgam was determined to reduce *L. monocytogenes* and *S. enteritidis* below the infectious dose when doner is moderately contaminated (~10^4 CFU/g). It should be investigated whether a larger amount of beverage consumption will be effective to eliminate the risk of *E. coli* O157: H7 completely, which has a very low infective dose and a high acid adaptation (38-40), in the case of moderate contamination.

Few studies have been performed to determine the effect of gastric fluid and food on bacterial survival. In a study conducted by Koseki et al. (1), it was determined that SGF has very little effect on *L. monocytogenes*; *E. coli* O157:H7, and Salmonella spp. with food (cabbage, tuna fish, hamburgers and eggs) intake without any beverage. Just and Daeschel (21) studied the effect of wine in SGF along with baby food and they determined the high inhibitory effect on *Salmonella typhimurim* and a little inhibitory effect on *E. coli* O157:H7. Similarly, this study demonstrated that consuming beverage with a meal may protect against some foodborne pathogens such as *S. enteritidis* and *L. monocytogenes* but not against others such as, *E. coli* O157:H7. To our knowledge, this is the first study to show the effect of beverage intake with food in a model stomach on pathogens.

Our study has several limitations; only one factor affecting the viability of the pathogens was checked and intestinal transit or its adhesive properties was not tested. Also, a comprehensive list of all available beverage and doner products has not been tested, generalizations about product lines are limited to tested products only.

In conclusion; the fact that some people, not everyone, who consumed the contaminated food, were poisoned during collective food poisoning cases; suggests that this is due to a difference in their personal immune system. Food safety of a popular fast-food such as doner kebab is a worldwide concern. There is no research on the effect of soft beverage consumption along with meat products that causes the main increase in stomach pH resulting in foodborne diseases. The data obtained in this study are expected to make an important contribution to the literature. This research shows that, the type and the amount of the beverage consumed along with food can be effective on foodborne illnesses in addition to the personal immune system. It can be concluded that consuming coke, ayran and especially shalgam while eating a suspicious food may decrease the risk of acute foodborne illness for a healthy person.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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