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The Effect of Keeping Time in Bain-Marie and Cooling Speeds on The Microbiological Quality of Meatballs Inoculated with *Escherichia coli*

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Abstract

This study aims to investigate the effect of keeping time in bain-marie and different cooling speeds on the microbiological quality of meatballs served in catering systems after contamination with *Escherichia coli.* For this purpose, the meatball mixture prepared was divided into two equal portions, Group A and B, and then subjected to frying, with Group B being inoculated with *E. coli*. After inoculation, the prepared meatballs were held at 70°C for 2 hours and cooled at different rates. The total mesophilic aerobic bacteria (TMAB) and yeast-mold counts in the meatball mixture, initially measured at levels of 5.59 log_{10} cfu/g and 7.91 log_{10} cfu/g respectively, decreased to levels of 2.92 log_{10} cfu/g and 3.58 log_{10} cfu/g after frying. After 2 hours in the bain-marie, the TMAB and yeast-mold levels in Group A meatballs were observed to be 2.42 log_{10} cfu/g and 2.30 log_{10} cfu/g, respectively. After inoculation, the TMAB, TAPB, *E. coli,* and yeast-mold levels in Group B meatball samples decreased continuously as a function of heating and cooling time. While the initial *E. coli* level was 8.13 log_{10} cfu/g, it decreased to 5.49 log_{10} cfu/g after 2 hours of heating and further decreased to 3.73 log_{10} cfu/g when the sample temperature reached 4°C. It was found that the microbial quality of Group B meatball samples cooled at +4°C was better than those cooled at room temperature. Meatball groups containing *E. coli* were found to have a higher pH value compared to those without *E. coli* and the pH increased further when both groups were left to cool at room temperature. The results suggest that applying rapid chilling after serving meatballs in catering systems positively affects the microbial quality of the product.

Key Words: Catering systems, cooling speed, *E. coli*, meatballs, microbiological quality

Escherichia coli ile İnokule Edilmiş Köftelerin Mikrobiyal Kalitesi Üzerine Benmaride Bekletme Süresi ve Soğutma Hızının Etkisi

Öz

Bu çalışma, toplu beslenme sistemlerinde servis edilen et köftelerinin *E. coli* ile kontaminasyonu sonrası ürünün mikrobiyolojik kalitesine benmaride bekleme süresi ve soğutma hızının etkisini araştırmayı amaçlamaktadır. Bu amaç için hazırlanan köfte hamuru A ve B grubu olarak iki eşit porsiyona ayrılarak kızartma işlemi uygulandı ve B grubuna *E. coli* inokulasyonu gerçekleştirildi. Hazırlanan köfteler benmaride 70°C'de 2 saat bekletildikten sonra farklı hızlarda soğutmaya tabii tutuldu. Köfte hamurunda sırasıyla 5.59 log_{10} kob/g ve 7.91 log_{10} kob/g seviyelerinde tespit edilen toplam mezofilik aerob bakteri (TAMB) ve maya-küf sayısı kızartma işlemi sonrasında 2.92 log_{10} kob/g ve 3.58 log_{10} kob/g seviyelerine indiler. Toplam aerob psikrofilik bakteri (TAPB) ve koliform sayısı köfte hamurunda sırasıyla 7.80 log_{10} kob/g ve 4.45 log_{10} kob/g iken kızartma işlemi sonrası bu mikroorganizmalar tespit edilemedi. Benmaride 2 saat bekleme sonrasında TAMB ve maya-küf düzeylerinin A grubu köftelerde sırasıyla 2.42 log_{10} kob/g ve 2.30 log_{10} kob/g olduğu gözlemlendi. İnokulasyon sonrası, B grubu köfte örneklerinin TMAB, TPAB, koliform ve maya-küf sayıları bekleme ve soğutma sürelerine bağlı olarak sürekli azalmıştır. *E. coli* miktarı başlangıçta 8.13 log_{10} kob/g iken, 2 saat benmaride bekletilmesinin ardından 5.49 log_{10} kob/g, örnek sıcaklığı 4°C'ye ulaştığında ise 3.73 log_{10} kob/g düzeyine inmiştir. Soğutması +4°C'de gerçekleştirilen B grubu köfte örneklerinin mikrobiyal kalitesinin, oda sıcaklığında soğutmaya bırakılanlara göre daha iyi olduğu saptandı. *E. coli* içeren köfte gruplarının içermeyenlere göre daha yüksek pH değerine sahip olduğu, her iki grubun oda sıcaklığında soğumaya bırakılması ile pH'ın daha da yükseldiği tespit edildi. Sonuçlar, toplu beslenme sistemlerinde sunulan köftelere servis sonrasında hızlı soğutma işlemi uygulanmasının ürünün mikrobiyal kalitesini olumlu yönde etkilediğini göstermiştir.

Anahtar Kelimeler: Catering sistemi, *E. coli*, köfte, mikrobiyal kalite, soğutma hızı

INTRODUCTION

To maintain a high quality of life and good health, people need an adequate amount of safe and nutritious food. Especially in today's world, catering systems are widely used in public/private industrial facilities, restaurants, schools, hotels, hospitals, and prisons (1). The number of people benefiting from catering systems is increasing every day, and almost every individual receives food services from these establishments at least once a day (2).

Insufficient attention to hygiene and sanitation regulations is a major problem in catering systems today. This not only affects the sensory qualities of food but also seriously threatens the health of consumers. Foodborne diseases cause deaths and significant economic losses (3,4). According to the World Health Organization (WHO), 1.6 million people worldwide fall ill every day due to unsafe food (5). Meat and meat products in particular are considered a risk to public health because of their high moisture content, nitrogenous components, and rich mineral content, which facilitate the vital activities of many microorganisms (6). In addition to the microbial spoilage associated with the growth of bacteria, yeasts, and molds in food, pH, an important parameter in this process, is also affected. Particularly in protein-rich meats and products, these microorganisms can cause an increase in meat pH by releasing alkaline nitrogen compounds such as ammonia and amines (7,8). To ensure food safety and quality, pH must be controlled. Escherichia coli, one of the bacteria that pose a threat to human health and cause foodborne illness, is known as an indicator of fecal contamination and belongs to the family Enterobacteriaceae (9). Contamination with this microorganism, found in the human and animal gastrointestinal systems in food establishments, seriously affects public health when contaminated equipment and water are used. Therefore, ensuring the traceability of meat and products, strict adherence to hygiene and sanitation rules, regular training of personnel, and delivery to consumers under a cold chain are crucial (9-11).

In addition to the hygiene regulations that apply to all stages from production to consumption in establishments that implement mass catering systems, attention must be paid to temperature control. Therefore, it has been stated in various sources that heat treatment applications should be below 5-10°C or above 65-70°C, and food should not be kept in this temperature range, also known as the danger zone, for more than 4 hours (12,13). When serving ready-to-eat meals in hot pot catering systems, the "double boiler" method is used to maintain the internal temperature of the food below 65°C for a maximum of 2 hours (14,15). Foods that are not to be consumed immediately should be subjected to rapid chilling (12).

The objective of this study is to monitor the microbial quality and pH changes of meatballs served in hot-pot catering systems during and after keeping time in bain-marie, depending on different cooling rates. Additionally, the study aims to investigate whether cooked meatballs reach the minimum infectious dose or not in the event of potential contamination with *E. coli* during this process.

MATERIAL AND METHODS

Procurement of Minced Meat and Spices

Minced meat containing 90% beef and 10% fat, to be used in the experimental production of meatballs, was obtained from butchers in Burdur province. The minced meat was transported to the laboratories of the Department of Nutrition and Dietetics, Faculty of Health Sciences, Burdur Mehmet Akif Ersoy University under cold chain conditions and stored at +4°C until the time of production. The spice mixture used in the production of meatballs was commercially purchased (Selay Meatball Seasoning, Istanbul).

Preparation of Meatball Mixture

The experimental production of meatballs was carried out in the laboratories of the Department of Nutrition and Dietetics, Faculty of Health Sciences, Burdur Mehmet Akif Ersoy University according to the method recommended by TS 10581. Meatballs were made by adding 8% meatball seasoning to ground beef, which contained 90% beef and 10% fat. After thorough mixing, the meatball mixture was portioned into balls with a diameter of 5 cm and a weight of 40-50 g each. These portions were then placed in sterile foam plates, covered with cling film, and stored in the refrigerator.

Determination of the Microbiological Quality of the Meatball Mixture

To determine the initial microbial load of the meatballs used in the study, a 10 g sample of the meatball mixture was weighed and placed in a sterile stomacher bag under aseptic conditions. Then 90 ml of peptone water was added and the mixture was homogenized using a stomacher. Serial dilutions of 1/10 were prepared and plated in duplicate on the appropriate media under the conditions described below. At the end of the incubation period, Petri dishes containing 30-300 colonies were evaluated and the results were expressed as $\log_{10}$ cfu/g.

Coliforms: Petri dishes inoculated with Violet Red Bile Dextrose Agar (Merck 110275) using the pour plate method were incubated at 37°C for 24 h (ISO, 2006).

E. coli: Detection was performed using Tryptone Bile X-Glucuronide Agar (Merck 116122). Petri dishes inoculated with the pour plate technique were incubated at 30°C for 4 h, followed by 44°C for 18 h (16).

Total mesophilic aerobic bacteria (TAMB): Plate Count Agar (Oxoid CM0325B) was used for plating using the pour plate method. Inoculated petri dishes were incubated at 37°C for 48 h (17).

Yeast and mold: Potato Dextrose Agar (Merck 110130) was used for plating using the spread plate method. Inoculated petri dishes were incubated at 25°C for 5 days (18).

Preparation of *E. coli* Bacterial Culture and Meatball Groups

The *E. coli* strain ATCC 29998 obtained from the bacterial culture collection of the Department of Nutrition and Dietetics, Faculty of Health Sciences, Burdur Mehmet Akif Ersoy University, was revived by inoculation on tryptic soy broth (TSB)

medium and incubated at 37°C for 24-48 hours. To contaminate the meatball samples, a dipping solution was prepared in physiological saline with a bacterial concentration of 10^8 CFU/mL.

The portioned meatballs were divided into two groups (groups A and B) after the cooking process. Group A was not inoculated with *E. coli*, whereas group B was inoculated with *E. coli* via the dipping solution. Group B meatballs were immersed in the bacterial suspension for 1 minute, ensuring thorough exposure of the entire surface through gentle rotation during the process.

Analyses Performed on Meatball Groups During the Hot-Hold and Cooling Processes

A sterile stainless-steel food holding container containing the meatball samples was placed in a double boiler heated to 70°C, and samples were taken at 0, 1, and 2 hours. After keeping time in a bain-marie for 2 h in the double boiler, the meatball groups were divided into two and kept at room temperature and refrigerator conditions. Sampling was performed when the internal temperature of the samples reached 25°C. Subsequently, all meatball samples were kept in the refrigerator until their internal temperature reached +4°C, and sampling was performed (Figure 1).

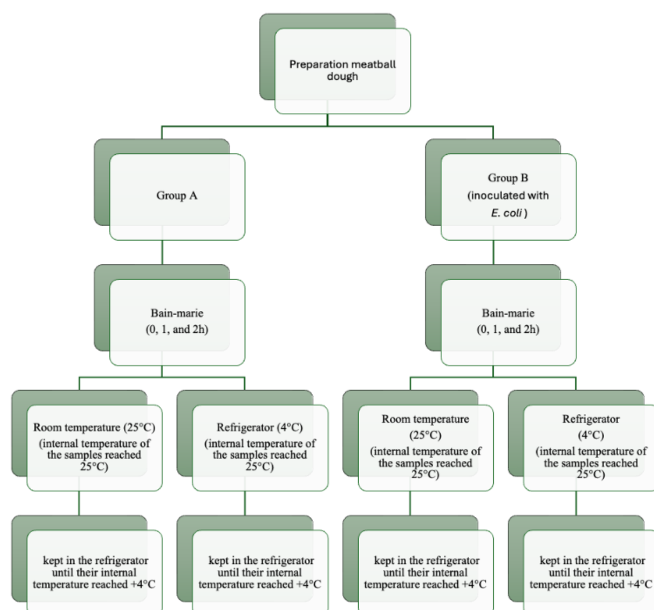

Figure 1. Preparation of groups and procedures applied

All samples were subjected to the following analyses.

Total mesophilic aerobic bacteria (TAMB): Plate count agar (Oxoid CM0325B) was used for plating using the pour plate method. Inoculated petri dishes were incubated at 37°C for 48 h (17).

Yeast and mold: Potato dextrose agar (Merck 110130) was used for plating using the spread plate method. Inoculated petri dishes were incubated at 25°C for 5 days (18).

Coliforms: Petri dishes inoculated with Violet Red Bile Dextrose Agar (Merck 110275) using the double layer pour plate method were incubated at 37°C for 24 h (16).

***E. coli*:** Detection was performed using Tryptone Bile X-Glucuronide Agar (Merck 116122). Petri dishes inoculated with the pour plate technique were incubated at 30°C for 4 hours, followed by 44°C for 18 h (19).

pH: The pH value of the meatball samples was determined using a digital pH meter (704 pH Meter, Metrohm) (20).

Statistical Analysis

The study was performed in triplicate. The results were analyzed using one-way analysis of variance (ANOVA) with Statistical Package for the Social Sciences (SPSS) (Version 25.0; SPSS, Chicago, IL, USA). To identify statistically significant differences, the Tukey test was used, and differences with $p<0.05$ were considered statistically significant. The relationship between pH and *E. coli* growth was evaluated using the Spearman correlation technique. Results are expressed as mean ± standard deviation (SD).

RESULTS

Microbiological Analysis Results

The levels of TAMB, TAPB, coliforms, and yeast-mold in the prepared meatball mixture were determined to be 4.59, 7.80, 4.45, and 7.91 $\log_{10}$ cfu/g, respectively. No TAPB and coliform bacteria were detected in Group A samples hot-held at different times after frying (Table 1). The TAMB count, which was 4.59 $\log_{10}$ cfu/g in the meatball mixture, decreased to 2.92 $\log_{10}$ cfu/g after the cooking process and further to 2.42 $\log_{10}$ cfu/g after the beginning of hot-hold time, 2.88 $\log_{10}$ cfu/g after the first hour, and 2.42 $\log_{10}$ cfu/g after the second hour ($p<0.05$). The yeast-mold count, which was 7.91 $\log_{10}$ cfu/g in the meatball mixture, decreased to 3.58 $\log_{10}$ cfu/g after cooking ($p<0.05$). It was found that Group A meatball samples hot-hold for 1 and 2 hours contained yeast-mold at levels of 2.61 and 2.30 $\log_{10}$ cfu/g, respectively, and the difference between the groups was statistically insignificant ($p>0.05$). No *E. coli* was detected in Group A meatball samples.

Table 1. Data obtained from the microbiological analysis of Group A meatball samples ($\log_{10}$ cfu/g) (Mean ± SD)

	TAMB	TAPB	Coliform	*E. coli*	Yeast-molds
Meatball dough	4.59±1.18^A	7.80±0.01	4.45±0.01	-	7.91±0.06^A
A0	2.92±0.04^B	-	-	-	3.58±0.12^B
A1	2.88±0.03^B	-	-	-	2.61±0.13^C
A2	2.42±0.07^C	-	-	-	2.30±0.27^C

A0: Group A Hot-hold 0 hours.; A1: Group A Hot-hold 1 hours.; A2: Group A Hot-hold 2 hours.
Groups labelled A, B and C are indicated by different letters within the same column to indicate significant differences. ($p<0.05$).

After the frying process, it was determined that the yeast-mold count of Group B meatball samples inoculated with *E. coli* was 8.21 log_{10} cfu/g at the initial placement in the double boiler (p<0.05). This count decreased to 8.09 log_{10} cfu/g after the first hour and further to 5.43 log_{10} cfu/g after the second hour (p<0.05). No psychrophilic microorganisms were detected in this group of meatballs after frying. As seen in Table 2, analyses conducted based on the meatball mixture and post-frying hot-hold times revealed a similar trend in coliforms and TAMB counts, with statistically significant differences observed in all values (p<0.05). It was observed that the level of *E. coli*, which was 8.13 log_{10} cfu/g at the beginning of the hot-hold in the double boiler, decreased to 5.49 log_{10} cfu/g after the second hour (p<0.05).

After 2 hours of hot-hold in the double boiler, all meatball groups were cooled to ambient (25°C) and refrigerator temperatures (4°C), and the microbiological data obtained are presented in Table 3 and Table 4.

Table 2. Data obtained from the microbiological analysis of Group B meatball samples (log_{10} cfu/g) (Mean ± SD)

	TAMB	TAPB	Coliform	*E. coli*	Yeast-molds
Meatball dough	4.59±1.18^{D}	7.80±0.01	4.45±0.01^{D}	-	7.91±0.06^{B}
B0	8.93±0.05^{A}	-	8.97±0.02^{A}	8.13±0.03^{A}	8.21±0.02^{A}
B1	8.32±0.05^{B}	-	8.88±0.03^{B}	8.00±0.03^{B}	8.09±0.08^{A}
B2	5.76±0.04^{C}	-	6.28±0.02^{C}	5.49±0.06^{C}	5.43±0.02^{C}

B0: Group B Hot-hold 0 hours.; B1: Group B Hot-hold 1 hours.; B2: Group B Hot-hold 2 hours.
Groups labelled A, B and C are indicated by different letters within the same column to indicate significant differences. (p<0.05).

Table 3. Microorganism levels of Group A meatball samples during cooling (log_{10} cfu/g) (Mean ± SD)

		Slow-cooling	Rapid- cooling	Sig. (2-tailed)
TAMB	A2 (70°C)	2.42±0.07^{B}	2.42±0.07^{A}	
	25°C	2.44±0.02^{B}	2.23±0.04^{B}	0.01
	4°C	2.97±0.04^{A}	2.38±0.04^{AB}	0.01
TAPB	A2 (70°C)	-	-	
	25°C	-	-	
	4°C	-	-	
Coliform	A2 (70°C)	-	-	
	25°C	-	-	
	4°C	-	-	
E. coli	A2 (70°C)	-	-	
	25°C	-	-	
	4°C	-	-	
Yeast-molds	A2 (70°C)	2.30±0.27^{AB}	2.30±0.27^{A}	
	25°C	1.19±1.04^{B}	2.08±0.08^{A}	0.25
	4°C	2.81±0.04^{A}	2.37±0.07^{A}	0.01

A2: Group A Hot-hold 2 hours.
Groups labelled A, B and C are indicated by different letters within the same column to indicate significant differences (p<0.05).

Table 4. Microorganism quantity of Group B meatball samples during cooling (log_{10} cfu/g) (Mean ± SD)

		Slow-cooling	Rapid- cooling	Sig. (2-tailed)
TAMB	B2 (70°C)	5.76±0.04^{A}	5.76±0.04^{A}	
	25°C	4.66±0.04^{B}	4.46±0.08^{B}	0.04
	4°C	3.24±0.04^{C}	3.01±0.02^{C}	0.01
TAPB	B2 (70°C)	-	-	
	25°C	-	-	
	4°C	-	-	
Coliform	B2 (70°C)	6.28±0.02^{A}	6.28±0.02^{A}	
	25°C	6.00±0.62^{B}	4.99±0.01^{B}	0.10
	4°C	4.79±0.08^{C}	4.21±0.10^{C}	0.01
E. coli	B2 (70°C)	5.49±0.06^{A}	5.49±0.06^{A}	
	25°C	4.51±0.05^{B}	4.15±0.01^{B}	0.01
	4°C	3.96±0.01^{C}	3.73±0.03^{C}	0.01
Yeast-molds	B2 (70°C)	5.43±0.02^{A}	5.43±0.02^{A}	
	25°C	4.73±0.05^{B}	4.70±0.02^{B}	0.20
	4°C	4.08±0.08^{C}	3.81±0.02^{C}	0.03

B2: Group B Hot-hold 2 hours.
Groups labelled A, B and C are indicated by different letters within the same column to indicate significant differences (p<0.05).

No TAPB, coliforms, or *E. coli* were detected in Group A meatballs. While TAMB levels were determined to be between 2.44-2.97 log_{10} cfu/g in meatballs first cooled to 25°C and then to 4°C in the ambient environment ($p<0.05$), these values were found to be 2.23 and 2.38 log_{10} cfu/g in those cooled to 25°C and 4°C in the refrigerator, respectively ($p>0.05$). In the yeast-mold analysis conducted under the same conditions, values of 1.19 and 2.81 log_{10} cfu/g were determined in ambient environment samples ($p<0.05$), while these values were 2.08 and 2.37 log_{10} cfu/g in refrigerator samples, respectively ($p>0.05$).

In the case of Group B meatball samples cooled to 25°C in ambient conditions, the TAMB count was at the level of 4.66 log_{10} cfu/g, while it decreased to 3.24 log_{10} cfu/g at 4°C ($p<0.05$). In the same group, for meatball samples cooled in the refrigerator, the TAMB count decreased from 4.46 log_{10} cfu/g at 25°C to 3.01 log_{10} cfu/g at 4°C ($p<0.05$). It was observed that the samples from Group B subjected to cooling processes did not contain TAPB. Both ambient and refrigerator samples of Group B showed the highest counts of coliforms, *E. coli,* and yeast-mold at 25°C, and the lowest counts at 4°C ($p<0.05$).

The pH of the samples

The pH values obtained from pH analysis conducted simultaneously with the microbiological analysis of the experimental meatball samples are presented in Table 5. The pH value, initially 6.43 in the meatball mixture, increased to 6.49 in Group A and 6.57 in Group B after the second hour of hot-hold ($p<0.05$). During cooling under ambient and refrigerator conditions, the pH values also increased in both groups. These changes were found to be statistically significant in the refrigerator samples of Group A and the ambient samples of Group B ($p<0.05$).

Table 5. pH values and *E. coli* quantity of Group A and Group B samples during marination and cooling processes (log_{10} cfu/g) (Mean ± SD)

	pH		pH
Meatball dough	6.43±0.02^{B}	Meatball dough	6.43±0.02^{B}
A0	6.47±0.03^{AB}	B0	6.48±0.01^{B}
A1	6.49±0.02^{A}	B1	6.48±0.03^{B}
A2	6.49±0.02^{A}	B2	6.57±0.03^{A}

	A			B		
	Slow-cooling	Rapid- cooling	Sig.(2-tailed)	Slow-cooling	Rapid-cooling	Sig. (2-tailed)
70°C	6.49±0.02^{A}	6.49±0.02^{B}		6.57±0.03^{C}	6.57±0.03^{A}	
25°C	6.52±0.01^{A}	6.52±0.01^{A}	1.00	6.63±0.01^{B}	6.56±0.02^{A}	0.01
4°C	6.53±0.03^{A}	6.54±0.01^{A}	0.37	6.68±0.02^{A}	6.60±0.02^{A}	0.01

A0: Group A Hot-hold 0 hours.; A1: Group A Hot-hold 1 hours.; A2: Group A Hot-hold 2 hours; B0: Group B Hot-hold 0 hours.; B1: Group B Hot-hold 1 hours.; B2: Group B Hot-hold 2 hours.
Groups labelled A, B and C are indicated by different letters within the same column to indicate significant differences. ($p<0.05$).

The meatball samples from Group A, which did not contain *E. coli*, showed a pH of 6.52 in both ambient and refrigerator conditions when cooled to 25°C, and a pH of 6.53 and 6.54, respectively, when cooled to 4°C. It was found that there was no statistically significant difference in the pH values of *E. coli*-inoculated Group B meatball samples when stored in refrigerator conditions ($p>0.05$); however, when kept at room temperature, an increase in pH was observed ($p<0.05$).

As seen in Table 6, the correlation coefficients indicate a strong relationship between the *E. coli* count and pH values in the samples of Group B that were subjected to 1 hour double boiler treatment, cooled to 25°C in ambient conditions, and reached 4°C in the refrigerator. For the other groups, there is a high degree of correlation between the data.

Table 6. Correlation between *E. coli* count and pH values in Group B samples. (log_{10} cfu/g) (Mean ± SD)

	E. coli	pH	r
B0	8.13±0.03	6.48±0.01	0.982
B1	8.00±0.03	6.48±0.03	-1.000
B2	5.49±0.06	6.57±0.03	0.998
Slow-cooling 25°C	4.51±0.05	6.63±0.01	-1.000
Slow-cooling 4°C	3.96±0.01	6.68±0.02	0.945
Rapid-cooling 25°C	4.15±0.01	6.56±0.02	-0.982
Rapid-cooling 4°C	3.73±0.03	6.60±0.02	1.000

B0: Group B Hot-hold 0 hours.; B1: Group B Hot-hold 1 hours.; B2: Group B Hot-hold 2 hours.

DISCUSSION AND CONCLUSION

In this study, the microbial and physicochemical properties of meatball samples were investigated using the double boiler technique commonly employed for the preservation of food until consumption in mass catering systems. Additionally, the study aimed to explore the development of microorganisms in meatball samples subjected to different

cooling rates to determine whether the minimum infectious dose would be reached in case of possible contamination.

Coliform microorganisms, commonly found in nature and indicative of fecal contamination, serve as essential indicators not only for hygiene but also for evaluating food safety and quality. When their numbers reach a certain level, they can cause off-flavors and shorten the shelf life of food products (21). Meat, during grinding, becomes more susceptible to microbial spoilage due to increased surface area exposed to air, creating an aerobic environment (22). According to Turkish food regulations, the total aerobic mesophilic bacteria (TAMB) content in ground meat and mechanically separated red meat should be between $5x10^5$-10^6 cfu/g, while *E. coli* should range from $5x10^1$-10^2 cfu/g. For meat mixtures, the *E. coli* content can be between $5x10^2$-10^3 cfu/g. Typically, food is considered spoiled when TAMB levels exceed 7 log CFU/g, and unpleasant odors may emerge when levels surpass 8 log_{10} cfu/g (23).

In a study investigating the effect of lavender essential oil on the physicochemical properties of meatballs throughout their shelf life, as well as its impact on microbiological quality, it was observed that the levels of TAMB, yeast, mold, and coliform in the prepared control groups were higher than the values found in this study (24). Another study on Tekirdağ meatballs reported TAMB and coliform counts in the meatball dough as 5.85 and 3.04, respectively (25). These variations in the meatball dough are believed to stem from differences in raw materials, equipment, and personnel.

Elverir and Gönülalan (26) evaluated microbial levels by sampling equipment, personnel, and food produced in a facility engaged in mass catering systems production to identify contamination sources. In a study where the TAMB and coliform counts in the prepared meatball dough decreased to $<1x10^2$ after cooking, it was reported that the food prepared after cooking did not pose a risk to public health if it was not subjected to any contamination.

Smith et al. (27) reported that cooking ground beef to an internal temperature of at least 71°C reduced the average probability of *E. coli* O157:H7 infection in Canada by a factor of $9.3x10^{10}$. During heat treatment processes, the danger zone is between 10°C and 65°C, and foods should not be kept at these temperatures for more than 4 hours (13). In catering systems, proper storage of cooked food at the right temperature and for the appropriate duration helps reduce the risk of foodborne infections. However, it does not provide complete protection against intoxications. Therefore, clear standard procedures should be established from raw material procurement to delivery to the consumer, with each step carefully monitored, and staff hygiene should be a priority (28).

In our study, microbial analysis of Group A meatball samples, which were not inoculated with *E. coli* after cooking, did not reveal the presence of TAMB, coliforms, or *E. coli*. Similarly, no total aerobic psychrophilic bacteria (TAPB) were detected in Group B samples. It is presumed that TAPB was inhibited during cooking. After keeping the Group B samples in bain-marie for 2 hours, a decrease of approximately 2.5 logs was observed in both coliform and *E. coli* counts. In a study conducted on meatball production in İzmir, TAMB levels were reported to be 4.89, 5.37, and 5.73 log_{10} cfu/g in minced meat, prepared minced meat, and raw meatball dough, respectively. Before shipment, the TAMB level in cooked Izmir meatballs was 0.86 log_{10} cfu/g, and in the hot-hold ones at the consumption point, it was 1.11 log_{10} cfu/g. No pathogenic microorganisms were detected in the same study (29). Considering these data, it is believed that if there is no contamination from personnel, keeping the food in a double boiler until it reaches the consumer will not deteriorate its microbial quality.

In catering facilities, not only is it crucial to cook meals at the correct temperature, but the serving and transfer stages are also critical. Dorman et al. (30) stated in their study that the primary cause of food poisoning incidents in these facilities is the reheating of cooked meals. The study emphasized the need to ensure the microbiological safety of cooked foods by serving them at a minimum temperature of 70°C and keeping those to be served cold at 5°C or below, and that foods not to be consumed immediately should undergo rapid pre-cooling (12).

E. coli is a microorganism that is resistant to various environmental conditions, including low pH and heat treatment. As a result, it is a significant contaminant in cooked ground beef. Although heat treatment should eliminate heat-labile *E. coli*, highly heat-resistant strains may persist and pose public health risks after storage (31). It is crucial to cool products quickly during food processing to avoid the growth of any remaining bacteria or the rejuvenation of spores, even when they are stored at appropriate temperatures. Despite being stored in a chilled environment, spore-forming bacteria (SFB) can still survive. Certain pathogenic SFBs, including *Clostridium botulinum*, *C. perfringens*, and *Bacillus cereus*, have been linked to food poisoning outbreaks in various foods, posing potential hazards in prepared chilled meals. Therefore, it is crucial to consider this as a critical production step (32). It is known that some food businesses intentionally disregard regulations to increase productivity during mass production. Several research investigations have demonstrated the significant role of chilling processes and their impact on food safety (32-34).

In the current study, it was observed that rapid cooling of Group B samples contaminated with *E. coli* resulted in lower counts of coliform, *E. coli*, and yeast-mold. This suggests that rapid cooling could improve the microbiological quality of the samples. Another study examined the microbial loads and pH values of meat and analogs contaminated with various pathogens stored at different temperatures and durations. The study discovered that storing beef at 4°C resulted in a maximum 2-log change in the initial total aerobic mesophilic bacteria (TAMB), coliforms, lactic acid bacteria (LAB), and yeast-mold counts. These counts did not show a significant increase until the 6th hour, particularly when stored at inappropriate temperatures (22-32°C). However, the 24 hour analysis reported a significant increase in coliforms and TAMB counts (34).

Changes in the pH of meat can be attributed to various factors, including microbial development. Studies suggest that lactic acid bacteria can cause a slight decrease in pH due

to the lactic acid compounds they produce, while the presence of non-lactic acid bacteria can cause a slight increase in pH. Additionally, microorganisms can release alkaline nitrogenous compounds such as ammonia and amines, which are known to increase the pH of meat (7,8). Both meatball samples showed an increase in pH after 2 hours of hot-hold, which is believed to be caused by TAMB and yeast-mold microorganisms. In the meat and analogs study, beef stored at 4°C exhibited a decrease in pH, while at 22°C and 32°C, there was a slight increase in pH at the 6th hour and a subsequent decrease at the 24th hour. The study found that the pH increased after 2 hours of hot-hold in both meatball samples, likely due to TAMB and yeast-mold microorganisms. Statistical significance was observed in the increase in pH in the groups of meatballs where *E. coli* was inoculated and left to cool at room temperature at different rates. In contrast, numerical increases in pH were observed in other meatball samples.

Controlling every stage, from raw material procurement to serving, is crucial in hot-pot catering systems. Incorrect implementation of these stages can result in both microbial and chemical spoilage of food, which can adversely affect public health. In our study, we simulated possible contamination from personnel or equipment after the cooking process. We found that hot-holding meatball samples inoculated with *E. coli* in a hot-pot resulted in approximately a 2.5-log reduction in both coliform and *E. coli* counts. This indicates the effectiveness of rapid cooling in positively impacting the microbial quality of the product and pH values indicative of microbial spoilage. Careful implementation of these procedures used in catering systems, along with staff training and attention to equipment hygiene, plays a crucial role in ensuring food safety and minimizing risks to public health.

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