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Growth of *Listeria monocytogenes* in çiğ köfte (raw meat ball)

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Summary: Çiğ köfte (raw meat ball), a traditional food of Turkey, has become a commercial success in recent decades. This study was undertaken to determine the growth of *Listeria monocytogenes* in çiğ köfte at 4 ºC and 25 ºC. For this purpose çiğ köfte samples were divided into four groups and contaminated with 10^3 cfu/g, 10^4 cfu/g and 10^5 cfu/g, respectively. Also there was a control group for detecting the contamination of *L. monocytogenes*. Samples were analyzed at 0, 4, 8, 12 and 24 hours by using Most Probable Number (MPN) Technique. The results were shown that there was a 1-2 log MPN/g increase within 8 hours in samples which were incubated at 25 ºC and also after 24 hours of incubation 2 log MPN/g increase were observed in all groups. In Group "A" *L. monocytogenes* level increased to 5.66 log MPN/g from the initial number of 2.97 log MPN/g within 24 hour. Similarly *L. monocytogenes* levels increased to 6.17 log MPN/g from 4.32 log MPN/g and 7.04 log MPN/g from the initial number of 5.17 log MPN/g in Group "B" and Group "C" within 8 hour period at 25 ºC, respectively. The results of this study indicate that if the contamination occur during the production process of çiğ köfte with *L. monocytogenes,* çiğ köfte should be considered as an important source of public health problems, even its initial contamination level is low. As the lack of legislation on çiğ köfte, it is necessary to establish microbiological standards by regarding the measures of European Community Standards also hygienic measures must be taken to ensure a wholesome product.

Key words: Çiğ köfte, *L. monocytogenes*, raw meat, spice.

Çiğ köftede *Listeria monocytogenes*'in gelişimi

Özet: Geleneksel bir ürün olan çiğ köfte son yıllarda daha çok ticari önem kazanarak geniş bir tüketim alanına sahip olmuştur. Bu çalışmada 4 ºC ve 25 ºC'lerde muhafaza edilen çiğ köftelerde *L. monocytogenes* gelişiminin belirlenmesi amaçlanmıştır. Çalışmada çiğ köfte örnekleri biri kontrol olmak üzere 4 gruba ayrılmıştır. Birinci grup 10^3 kob/g, ikinci grup 10^4 kob/g ve üçüncü grup 10^5 kob/g düzeyinde *L. monocytogenes* (NCTC-2167) ile kontamine edildikten sonra 0., 4., 8., 12. ve 24. saatlerde USDA/FSIS tarafından önerilen yöntem ile çiğ köftelerdeki *L. monocytogenes* gelişimi analiz edilmiştir. *L. monocytogenes*'in düzeyinin belirlenmesinde ise Most Probable Number (MPN) tekniği kullanılmıştır. Çalışmada 25 ºC'deki üç grupta da ilk 8 saat içerisinde 1-2 log MPN/g düzeyinde artış olduğu, 24 saat sonunda ise bu artışın 2 log MPN/g düzeyine ulaştığı saptanmıştır. Bu kapsamda A grubunda 2.97 log MPN/g olan başlangıç kontaminasyon düzeyi 25 ºC'de 24 saat sonunda 5.66 log MPN/g seviyesine yükselmiştir. B ve C gruplarında ise sırasıyla 4.32 log MPN/g ve 5.17 log MPN/g'dan 8. saat sonunda 6.17 log MPN/g ve 7.04 log MPN/g düzeyine ulaşmıştır. Çalışma bulgularından elde edilen sonuçlar ışığında yetersiz hijyenik koşullar altında üretilen çiğ köftelerde düşük düzeyde dahi *L. monocytogenes* ile bir kontaminasyon durumunda halk sağlığı riski oluşabileceği bu nedenle üretimden tüketime kadar her aşamada asgari hijyenik şartlar ile uygun muhafaza koşullarının sağlanması gerektiği düşünülmektedir. Bu amaca yönelik olarak, Türkiye'de çiğ köftelerin de yer aldığı hazır gıdalara yönelik yasal düzenlemelerin Avrupa Birliği mevzuatı dikkate alınarak hazırlanmasının halk sağlığı ve gıda güvenliği açısından yararlar sağlayabileceği görüşüne varılmıştır.

Anahtar sözcükler: Baharat, çiğ et, çiğ köfte*, L. monocytogenes*.

Introduction

Çiğ köfte is a special food in Turkey which is prepared from raw, nonfat ground beef, onion, pounded wheat (bulgur) and different spices such as, red and black pepper, cumin and allspice, salt and tomato paste by mixing with hands without any heating process. Çiğ köfte has become a commercial attribute and sold in streets and supermarkets as a traditional ready-to-eat food from the last decade. Because of its properties, which is consumed raw, çiğ köfte have to be eaten in 1 or 2 hour but in the markets this period extend up to 24 hour. Although it is commonly consumed in Turkey, there is no specific standard regarding the level of microbiological quality, ingredients or manufacturing technique (5,8,13,18).

Çiğ köfte possess potential risk as a public health according to the studies taken to determine the microbiological quality of its ingredients such as raw minced beef and spices. *Salmonella* spp., *L. monocytogenes*, *S. aureus*, *C. perfringens*, *E. coli*

O157:H7, *Y. entereocolitica* and *B. cereus* are the important pathogens in raw minced beef and spices so it express that çiğ köfte can be easily contaminated by this bacterial pathogens (9,13,15,19,23). Among these pathogens *L. monocytogenes* is ubiquitous and can grow at pH levels between 5.0 and 9.6, also *L. monocytogenes* 1/2a and 4b is the major serotypes which have 30 % mortality in consumption of contaminated food (10, 17, 20). Despite the fact that a wide variety of foods may be contaminated with *L. monocytogenes*, outbreaks and sporadic cases of listeriosis appear associated with ready-to-eat (RTE) products. It is considered that post processing treatments, raw meat and vegetables, storage period and conditions, exceeding storage time are all increasing factors for the contamination risk of RTE with *L. monocytogenes* (4). As a result of previous reports raw minced beef samples have a contamination level of *Listeria* spp. with a ratio of 39-100 %, among them 15-80 % were contaminated with *L. monocytogenes* and also it is indicated that contamination will be increased by post processing treatments (6,12,21,22,25). Conspicuously in Turkey, it is remarked that the contamination level of raw minced beef with *Listeria* spp. were 56-97 % and 23-40 % with *L. monocytogenes* (7,16,24).

According to their regulations and directives in member of European Union and many other countries, *L. monocytogenes* have zero tolerance in RTE foods. Beside the ability to survive for long periods of time in the environment and on foods and in food processing plants, and its ability to grow at very low temperatures (0 ºC to 4 ºC) have made *L. monocytogenes* a major concern for the food hygiene (1,2). Thus, the specific aim of this experimental study is to determine the growth activity of *L. monocytogenes* in çiğ köfte with different storage temperature and to expose the potential risks of listeriosis as mentioned above.

Materials and Methods

Çiğ köfte production and experimental design of groups

All the ingredients were detected for the contamination of *L. monocytogenes.* In order to prepare çiğ köfte ingredients (spices, onion, garlic, parsley, ground beef as shown on Table 1) were molded with sterile distilled water. After adding raw minced beef the mixture molded for becoming doughy. Çiğ köfte samples were divided into 4 groups, each group consist of 500 g çiğ köfte; Group K, control group which was not contaminated with *L. monocytogenes*; Group A, contaminated with *L. monocytogenes* at a level of 10^3 cfu/g; Group B, contaminated with *L. monocytogenes* at a level of 10^4 cfu/g; Group C, contaminated with *L. monocytogenes* at a level of 10^5 cfu/g. All samples were incubated at 4 ºC and 25 ºC in sterile stomacher bag after the inoculation step. Samples were tested for *L. monocytogenes* counts at 0, 4, 8, 12 and 24 hours of incubation by using Most Probable Number Technique (MPN). The experimental application was repeated two times.

Table 1. Amounts of ingredients in experimental çiğ köfte production (g / 1kg).
Tablo 1. Deneysel çiğ köfte yapımında kullanılan malzemeler ve miktarları (g / 1kg).

Ingredient	Amount
Pounded wheat	550
Ground meat	360
Onion	10
Tomato paste	10
Red pepper	15
Spring onion	15
Parsley	15
Water	15*
Allspice	5
Black pepper	2
Salt	2
Garlic	1

* ml / 1kg

Preperation of bacterial culture

L. monocytogenes serotype type 1 (NCTC-2167) was provided from Refik Saydam Laboratory Culture Collection, Ankara – Turkey. The culture was firstly inoculated to Tryptone Soya Broth (Oxoid, CM 129) and incubated at 37 ºC for 24 hour for activation. Process culture suspension was homogenized and diluted with 0.1 % sterile peptone water to 10^{-8} decimal dilutions and inoculated to Tryptone Soya Agar (Oxoid CM131) – Yeast Extract (Oxoid L21) (TSA-YE) and Modified Oxford Agar (MOX) (Merck 1.07004) for bacterial enumeration. Plates were incubated at 37 ºC for 24 hour. Then culture was diluted with sterile peptone water (0.1 %) and were aseptically transferred into 500 g çiğ köfte to obtain a final inoculums of approximately 5.0 x 10^3 cfu/ml, 5.0 x 10^4 cfu/ml, 5.0 x 10^5 cfu/ml for Group A, B and C, respectively.

L. monocytogenes isolation and identification procedure

The contamination level of samples was detected by using the method of United States Department of Agriculture (USDA) – Food Safety and Inspection Service (FSIS) (14). Bacterial counts were determined by using Most Probable Number Technique (MPN). For each treatment 10g, 1g and 0.1 g of çiğ köfte samples were inoculated to Modified University of Vermont *Listeria* Selective Enrichment Broth (Merck, 1.10824)

(UVM) for primary enrichment. After incubation 0.1 ml UVM enrichment were transferred to 10 ml of Fraser *Listeria* Selective Enrichment Broth (Base) (FB) for secondary enrichment step. Inoculated FB tubes were incubated at 35 ºC for 24-48 hour. Then FB were inoculated into Modified Oxford Agar (MOX) (Merck 1.07004) and incubated at 35 ºC for 24-48 hour. Typical *L. monocytogenes* colonies were also tested for Gram stained, activity on SIM Medium (Oxoid CM 0435), catalase and hemolysin tests for confirmation. The number of tubes per dilution that were ultimately found to be *L. monocytogenes* positive determined by using 3-tube MPN table (3).

Statistical analyses

The results obtain in this study were analyzed using cubic regression model. For this purpose clarification coefficient of Group A is assigned as 0.997, 0.954 for Group B and 0.927 for Group C (SPSS, 11.5 version, ref no: 651544.).

Cubic Regression Model; Group A, y= 2.9528+ 0.2323x(hour)-0.0193 x(hour ^{2})+0.0006 x(hour ^{3}); Group B, y= 4.2402+0.0739 x(hour)+0.0224x(hour ^{2})-0.0009 x(hour ^{3}); Group C, y= 5.0854+0.2235 x(hour)-0.0023 x(hour ^{2})-0.0001 x(hour ^{3}).

Results

The progress of *L. monocytogenes* in çiğ köfte samples which were incubated at 25 ºC were remarked on figure 1, 2 and 3. In Group "A" *L. monocytogenes* level increased to 5.66 log MPN/g from the initial number of 2.97 log MPN/g within 24 hour. Similarly *L. monocytogenes* levels increased to 6.17 log MPN/g from 4.32 log MPN/g and 7.04 log MPN/g from the initial number of 5.17 log MPN/g in Group "B" and Group "C" within 8 hour period, respectively. Likewise the statistical evaluation of results in time showed a curvilinear increase. So high level of clarification coefficient showed the increase according to time was explained sufficiently by statistically.

Dissimilarly, there was no increase in the level of *L. monocytogenes* in all three group of samples which were incubated at 4 ºC.

Discussion and Conclusion

It is observed that all contaminated çiğ köfte sample groups which were incubated at 25 ºC have a 2 log increase from the initial contamination levels within 24 hour. However there was no increase in contamination level of same groups which were incubated at 4 ºC at the same time. Also it is conspicuous that there was a rapid increase of *L.monocytogenes* within 8 hour as shown in figures 2 and 3 in group B and C.

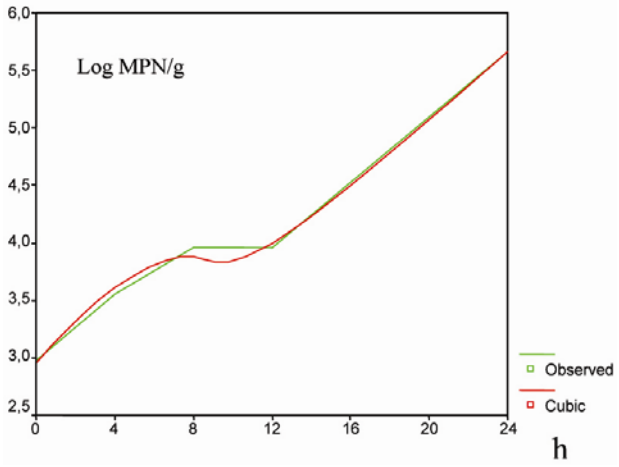

Figure 1. Progress of *L. monocytogenes* at 25 ºC in contaminated çiğ köfte samples (Group A – 10^{3} cfu/g).
Şekil 1. 10^{3} kob/g düzeyinde kontamine edilen, çiğ köfte örneklerinde *L. monocytogenes*'in gelişim seyri (25 ºC – Grup A).

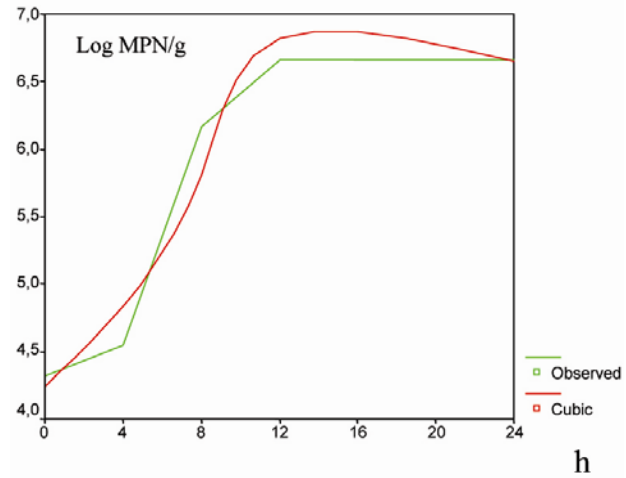

Figure 2. Progress of *L. monocytogenes* at 25 ºC in contaminated çiğ köfte samples (Group B – 10^{4} cfu/g).
Şekil 2. 10^{4} düzeyinde kontamine edilen, çiğ köfte örneklerinde *L. monocytogenes'in* gelişim seyri (25 ºC – Grup B).

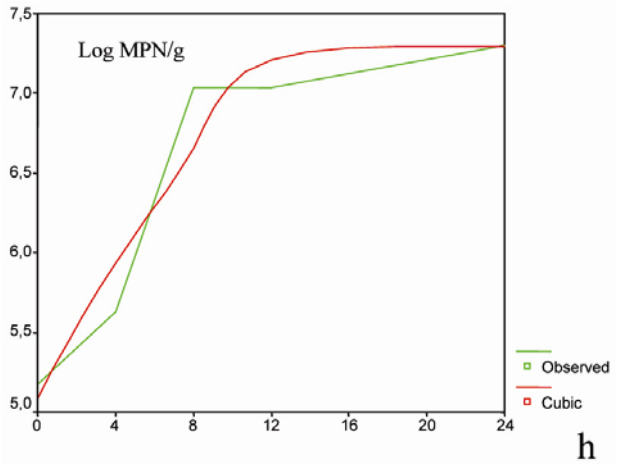

Figure 3. Progress of *L. monocytogenes* at 25 ºC in contaminated çiğ köfte samples (Group C – 10^{5} cfu/g).
Şekil 3. 10^{5} düzeyinde kontamine edilen, çiğ köfte örneklerinde *L. monocytogenes'in* gelişim seyri (25 ºC – Grup C).

Arslan et. al. (5) found that the level of hygiene index microorganisms of çiğ köfte sold in supermarkets were 10^3 – 10^6 cfu/g. Likewise, Küplülü et al (13) detected that the number of *Enterobacteriaceae*, coliform and enterococci in çiğ köfte samples that sold in Ankara as 4.39 $\log_{10}$cfu/g, 4.24 $\log_{10}$cfu/g and 4.39 $\log_{10}$cfu/g, respectively similarly with the results of Sağun et al (18). Even these studies were conducted in different region of Turkey, the results show that hygienic qualities of çiğ köfte is poor and has a health risk potential for the consumers because there is lack of good manufacturing practices at production to outlets line. Alike, İşleyici et. al. (11) determined the *Listeria* spp. from çiğ köfte samples sold in Van province markets. Ten of 50 (20 %) çiğ köfte samples were contaminated with *Listeria* spp. and 2 % were found to be *L. monocytogenes*. Şireli and Erol (24) isolated different *Listeria* spp. from 97 % of the 100 minced beef samples at the level of 0.23 – 2.9 x 10^3 MPN/g. The contamination of *L.monocytogenes* in the same samples was determined to be 0.72 – 2.9 x 10^3 MPN/g. Also these results support our findings and point out that there is a high potential risk in çiğ köfte even there is a low contamination level of *L.monocytogenes* if there is an inadequate hygienic condition in the production process and in retail markets.

As a matter of fact, listeriosis is not known whether the differences in incidence rates between developed and developing countries reflect true geographical differences, differences in food habits and food storage. Also it is clear that differences in traditional food consumption, hygienic quality of these foods in production line and storage period have arise the risk factors in listeriosis (4).

Consequently, we determined that çiğ köfte as a ready-to-eat food may be easily contaminated by the ingredients in production (raw minced beef, spice etc.). Also factors such as inappropriate handling, lack of personnel hygiene, maintaining çiğ köfte at room temperatures at the points of sale, especially in summertime, short shelf life, and inadequate hygienic conditions may cause serious health problems.

As a result of this study the official controls must be taken at points of sale and also cold chain has to be carried out at production and sale line. Hazard Analysis and Critical Control Points (HACCP) and Good Manufacturing Practices (GMP) must be put into practice with optimum cleaning and disinfection at production line of çiğ köfte. Also it is important that there must be legislation on microbiological quality and manufacturing technique of çiğ köfte.

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