

Thermal Resistance of *Listeria monocytogenes* in İnegöl Meatballs

G. Ece SOYUTEMİZ, Figen ÇETİNKAYA*

Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Uludağ University, 16059 Görükle, Bursa- TURKEY

*E-mail: figcetinkaya@yahoo.com

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Abstract: This study was performed to determine the thermal resistance of *L. monocytogenes* at different heat treatments in İnegöl meatballs. İnegöl meatballs were divided into three groups. In group I, meatballs were inoculated with 8.0×10^2 , 1.9×10^3 , 6.0×10^3 and 2.4×10^4 cfu/g *L. monocytogenes* and then they were cooked at an internal temperature of 85 °C for 4 min. Group II meatballs were inoculated with 1.0×10^2 , 2.0×10^2 , 8.0×10^2 , 5.6×10^5 and 2.0×10^6 cfu/g *L. monocytogenes* and cooked at an internal temperature of 80 °C for 4 min. In group III, the meatballs were inoculated with 5.6×10^5 and 2.0×10^6 cfu/g *L. monocytogenes* and cooked at an internal temperature of 63 °C for 30 min. In groups I and II, *L. monocytogenes* was not determined by direct plating method after cooking process. However, heat injured bacteria were recovered by an enrichment procedure when they were cooked at an internal temperature of 80 °C for 4 min. In group III, *L. monocytogenes* numbers were reduced by about 3 log cfu/g at 63 °C for 30 min. As a result, it was considered that the cooking process at an internal temperature of 85 °C for 4 min of İnegöl meatballs is sufficient for eliminating *L. monocytogenes* when it is at $\leq 10^4$ cfu/g level, and the heat treatment at 80 °C for 4 min was not adequate to completely eliminate the pathogen when its number was higher than 10^2 cfu/g but it decreased the *L. monocytogenes* levels to undetectable numbers. However, it was concluded that the cooking process carried out at 63 °C for 30 min did not entirely eliminate the high contamination ($\geq 10^5$ cfu/g) of *L. monocytogenes*, and the meatballs may constitute a risk for foodborne listeriosis, especially in susceptible human hosts.

Key Words: İnegöl meatball, *Listeria monocytogenes*, heat treatment

İnegöl Köftelerde *Listeria monocytogenes*'in Isıya Direnci

Özet: Bu çalışma, farklı ısı uygulamalarına maruz bırakılan İnegöl köftelerde *L. monocytogenes*'in ısıya direncini saptamak amacıyla gerçekleştirildi. İnegöl köfteler üç gruba bölündü. Birinci grup köfteler 8.0×10^2 , 1.9×10^3 , 6.0×10^3 ve 2.4×10^4 kob/g düzeylerinde *L. monocytogenes* ile inokule edildi ve daha sonra 85 °C'lik iç ısıda 4 dakika süreyle pişirildi. İkinci grupta, köfteler 1.0×10^2 , 2.0×10^2 , 8.0×10^2 , 5.6×10^5 ve 2.0×10^6 kob/g seviyesinde *L. monocytogenes* ile inokule edilerek 80 °C iç ısıda 4 dakika süreyle pişirildi. Üçüncü grup köfteler ise 5.6×10^5 ve 2.0×10^6 kob/g düzeyinde *L. monocytogenes* ile inokule edilerek, iç ısı 63 °C'de 30 dakika süreyle pişirildi. Birinci ve ikinci gruplarda, pişirme işlemi sonrası direk ekim yöntemiyle *L. monocytogenes* saptanmadı. Ancak, 80 °C iç ısıda 4 dakika pişirme işlemi gören köftelerde ısıya zarar görmüş bakteriler zenginleştirme işlemiyle belirlendi. Üçüncü grupta, *L. monocytogenes* sayıları 63 °C'de 30 dakika süreyle uygulanan pişirme işlemiyle yaklaşık 3 log kob/g azaltıldı. Sonuç olarak, İnegöl köftelere 85 °C'lik iç ısıda 4 dakika süreyle uygulanan pişirme işleminin $\leq 10^4$ kob/g seviyelerindeki *L. monocytogenes*'in eliminasyonu için yeterli olduğu ve 80 °C'de 4 dakikalık ısı uygulamasıyla 10^2 kob/g'in üzerindeki patojen sayılarının tam olarak yıkılmadığı, yalnızca belirlenemeyen seviyelere azaldığı görüldü. Bununla birlikte, 63 °C'de 30 dakika süreyle uygulanan pişirme işleminin *L. monocytogenes*'in yüksek kontaminasyonunu ($\geq 10^5$ kob/g) tamamen elimine etmediği ve bu köftelerin gıda kaynaklı listeriosis açısından, özellikle duyarlı kişilerde, risk oluşturabileceği sonucuna varıldı.

Anahtar Sözcükler: İnegöl Köfte, *Listeria monocytogenes*, ısı işlemi

Introduction

Listeria monocytogenes, a Gram positive, aerobic to facultative anaerobic, nonsporulating intracellular pathogen, has the potential to cause human listeriosis. This organism is widely spread throughout the environment and has been isolated from various sources, including food, soil, sewage, animal feed, and decaying

vegetation (1,2). Food is the primary means of transmission of this pathogen. Previous studies have shown that *L. monocytogenes* is frequently isolated from soft cheeses (3-5), milk (6,7), fish products (8), and meat and poultry products (9-12).

A wide variety of meats are contaminated with *L. monocytogenes* with the incidence of contamination (cfu

per gram) varying greatly (2,13). The prevalence of *L. monocytogenes* in meats ranges from 0% to 92% (13), with the overall prevalence of the pathogen for meats estimated at 16% (14). Johnson et al. (15) found *L. monocytogenes* in the interior muscle cores of 5 of 110 total samples of beef, pork and lamb roasts. These organisms were probably present in the muscle at the time of slaughter. For minced meats, several prevalence rates ranging from 11% to 69% have been reported (16,17).

Although much of the previous work on the thermal resistance of *L. monocytogenes* has been performed with dairy products, some work has recently been done on the heat resistance of *L. monocytogenes* in meat and meat products. Some interesting points that have been found include the following: a) although the addition of beef fat does not appear to enhance the heat resistance of *L. monocytogenes*, the presence of curing salt substantially increases it, b) the heat shock phenomenon must be taken into account when heating meats, especially for products heated slowly to a final internal temperature (18-20).

This study was carried out in order to determine the thermal destruction of *L. monocytogenes* in İnegöl meatballs. This meat product was chosen since it is a well-known meat dish in most parts of Turkey, especially in Bursa.

Materials and Methods

Preparation of İnegöl meatballs

İnegöl meatballs were provided from a local restaurant in Bursa province (Turkey). Briefly, preparing stages of İnegöl meatballs were as follows:

A mixture of veal (71%) and lamb (12%) meats was passed through a grinding machine and used for the preparation of meatballs. It is important to note that the fat part of the meats and also rib fat parts were included in the mixture. Firstly salt (1%), sodium bicarbonate (0.6%) and crumbled stale bread (6.2%) were added to the mixture and the result was thoroughly mixed for 10-15 min in a mixer specially made for this purpose before passing again through the grinding machine. Thereafter onion at the ratio of 8.5% was added to the meatball dough and this was again subjected to mixing for 10-15 min. Finally, the meatball dough was shaped by means of İnegöl meatball forming equipment.

Bacterial strains and preparation of inoculum

L. monocytogenes strain SLCC 2375 serotype 4b used in this study was kindly provided by Dr. Ch. Jacquet, Institut Pasteur, and Center National de Reference des Listeria, Paris, France. Stock cultures were maintained on Tryptone Soya Agar (TSA) (Oxoid, CM 131) slants at 4 °C and subcultured monthly. The cultures for experiments were prepared from a stock culture by inoculating Tryptone Soya Broth (TSB) (Oxoid, CM 129), which was then incubated at 35 °C for 24 h. This TSB culture was surface plated onto Oxford agar (Oxoid CM856 + SR140) and incubated at 35 °C for 24 h. After a typical colony (producing black zones) was transferred to 10 ml of TSB with incubation at 35 °C, further serial dilutions from 10^{-1} to 10^{-6} were prepared by using 9 ml sterile peptone water at 1st, 2nd, 3rd, 4th, 5th and 6th h of the incubation. These dilutions were used to enumerate *L. monocytogenes* by surface-plating 0.1 ml of Oxford agar. The plates were incubated at 35 °C for 24 to 48 h, after which all the characteristic visible colonies were counted and so levels of *L. monocytogenes* in 1 ml of TSB were determined.

Inoculation of the meatballs with *L. monocytogenes*

The meatballs were firstly tested for the presence of *L. monocytogenes* before inoculation and no pathogen could be detected. After confirming the samples as pathogen free, the meatballs were manually kneaded in a sterile container before inoculation and then weighed aseptically into 400 g, 500 g and 200 g portions for inoculation of the group I, II and III meatballs, respectively. Thereafter each portion was divided aseptically into portions of 100 g. The portions of grup I of meatballs were inoculated with 8×10^2 cfu/g, 1.9×10^3 cfu/g, 6.0×10^3 cfu/g and 2.4×10^4 cfu/g *L. monocytogenes*. Group II meatballs were inoculated with *L. monocytogenes* to have a final concentration of 1×10^2 cfu/g, 2×10^2 cfu/g, 8×10^2 cfu/g, 5×10^5 cfu/g and 2×10^6 cfu/g. Group III meatballs were inoculated with *L. monocytogenes* at levels of 5.6×10^5 cfu/g and 2.0×10^6 cfu/g. Following manual mixing, each inoculated portion was manually formed into patties (ca. 5 cm length and 1.5 cm width by 15 g weight) using approximately uniform pressure. The meatballs were then subjected to a cooking process. For each inoculation level and heat treatments three parallel replicates have been made and then counts from three parallel samples were averaged for a given inoculation level.

Cooking of the meatballs

A temperature-time controlled equipment (EMKO A.Ş.) for meat products was used during cooking of meatballs and the meatball centre temperature was measured via a platinum resistance thermometer (PT-100, IEC751 Class B) in this unit. The meatballs were placed in the equipment when the internal temperature was at 120 °C. Group I, II and III meatballs, after a platinum resistance thermometer had been inserted, were cooked separately at internal temperatures of 85 °C for 4 min, 80 °C for 4 min and 63 °C for 30 min, respectively. The time and temperature up shift correlation for both of group I and II samples was as follows: from 65 °C to 70 °C in 10 min and from 70 °C to 80 °C in 15 min. Only for the group I a supplementary 10 min was required to reach 85 °C. For the group III meatballs, the up shift was 15 min from 50 °C to 60 °C and 5 min from 60 °C to 63 °C. At the end of cooking process, the meatballs were immediately placed directly into stomacher bags, kept at 0 °C for fast cooling and analysed as soon as possible.

Microbiological analysis

For enumeration of *L. monocytogenes*, a 25-g sample from each group meatball was homogenised in 225 ml of 0.1% peptone water for 2 min; then 0.1 ml portions of an appropriate dilution were spread on the surface of Oxford agar. The plates were incubated at 30 °C for 48 h, after which all the characteristic visible colonies

(exhibiting a grey-black colour with a black halo) were counted. Representative colonies (five per plate) were confirmed as *L. monocytogenes*, based on morphology, catalase production, beta-haemolysis, tumbling motility, oxidase test, xylose, rhamnose and mannitol fermentation. Furthermore, cooked samples in which *L. monocytogenes* was not detected by direct plating were subjected to enrichment procedure. This included incubation of 25 g meatball samples in 225 ml Tryptone Soya Broth + 0.6 % Yeast Extract (YE, Oxoid, L21) at 35 °C for 24-48 h. Enrichment culture was then plated on Oxford agar plates and all plates were incubated at 37 °C for 48 h. Following above mentioned confirmation tests, characteristic colonies were identified as *L. monocytogenes* (21).

Results

The survival of *L. monocytogenes* at different cooking temperatures in İnegöl meatballs is given in the Table. *L. monocytogenes* was not detected in the meatballs inoculated with 8×10^2 , 1.9×10^3 , 6×10^3 or 2.4×10^4 cfu/g bacteria after heat treatment at an internal temperature of 85 °C for 4 min of the meatballs. *L. monocytogenes* counts decreased to undetectable levels in the meatballs with inoculum at the levels of 1×10^2 , 2×10^2 , 8×10^2 , 5.6×10^5 and 2.0×10^6 cfu/g and cooked at an internal temperature of 80 °C for 4 min. But in these meatballs, *L. monocytogenes* could be detected after the enrichment

Table. Survival of *L. monocytogenes* at different cooking temperatures in İnegöl meatballs.

| Groups | Internal temperature of the meatballs (°C) | Cooking time at internal temperature (min) | Inoculum levels of meatballs (cfu/g) | <i>L. monocytogenes</i> counts after cooking (cfu/g) | <i>L. monocytogenes</i> positive samples after enrichment procedure |
|--------|--|--|--------------------------------------|--|---|
| I | 85 | 4 | 8.0×10^2 | - | - |
| | 85 | 4 | 1.9×10^3 | - | - |
| | 85 | 4 | 6.0×10^3 | - | - |
| | 85 | 4 | 2.4×10^4 | - | - |
| II | 80 | 4 | 1.0×10^2 | - | + |
| | 80 | 4 | 2.0×10^2 | - | + |
| | 80 | 4 | 8.0×10^2 | - | + |
| | 80 | 4 | 5.6×10^5 | - | + |
| | 80 | 4 | 2.0×10^6 | - | + |
| III | 63 | 30 | 5.6×10^5 | 6.0×10^2 | + |
| | 63 | 30 | 2.0×10^6 | 1.6×10^3 | + |

process. In the meatballs that had 5.6×10^5 and 2.0×10^6 cfu/g initial *L. monocytogenes* levels, the heat treatment at an internal temperature of 63 °C for 30 min decreased the numbers of bacteria to 6×10^2 and 1.6×10^3 cfu/g, respectively.

Discussion

In this study no viable *L. monocytogenes* was detected in the group I and II of meatballs cooked at an internal temperature of 85 °C and 80 °C for 4 min, respectively. However, in group II, the pathogen could be detected after the enrichment process due to the possible the recovery of the injured cells. In group III, approximately a 3 log cfu/g decrease was observed in *L. monocytogenes* counts after heat treatment.

There are several studies on heat destruction of *L. monocytogenes* in different meat products and death time of the pathogen is varying depending upon the environment nutritional factors. Carpenter and Harrison (22) inoculated boned chicken meat with 10^5 - 10^7 /g cultures of *L. monocytogenes* and subsequently heated in a microwave and found that the bacteria survived in the thermally treated samples at an internal temperature of 65.6-82.2 °C. Boyle et al. (23) reported that death times of bacteria in the meats at 60, 65 and 75 °C were 2.54, 0.75 and 0.23 minutes, respectively. Gaze et al. (24) investigated the thermal resistance of *L. monocytogenes* in chicken, beef and carrots at a temperature of 60-70 °C. They pointed out that the bacteria could be eliminated if the meat is heated at 70 °C for 2 min. Coote et al. (25) found that heating for the full cook time of 28 min resulted in a mean measured temperature of 85 °C and no surviving *Listeria* were detected on chicken skin. This indicated eradication in viable numbers of greater than 10^6 . Sofos et al. (26) determined that when the ground pork products were cooked to 67 °C destruction of *L. monocytogenes* exceeded 6 log cfu/g in uncured as well as cured treatments. Other examples for meats include a D_{145F} of 0.47 to 0.73 in naturally contaminated ground beef and 2.56 to 2.82 in ground roast beef (27).

Karaianoglou and Xenos (28) searched thermal resistance of grilling process at 110-120 °C for 15 min

in meatballs and determined that meatballs did not have *L. monocytogenes* after those internal temperatures. The results observed at 63 °C for 30 min in the present study are in general agreement with results previously reported for the pathogen in summer sausages (29). Palumbo et al. (30) studied the inactivation of *L. monocytogenes* during the thermal processing of liver sausage. They have found that in product heated to 145°F (63 °C), the number of viable *L. monocytogenes* decreased and at 155°F (68 °C), no viable *L. monocytogenes* were detected.

The results of different studies indicate the existence of considerable variations among the reports on the heat resistance of *L. monocytogenes*. There are several factors altering the level of heat resistance of *L. monocytogenes* in the reported studies, such as differences among the strains, inoculum level, preparation of the product, substrate specific effects, experimental condition, protocols, recovery media and methods. Thus, direct comparisons between the studies are difficult, although it is reasonable to accept that at least some of these factors underlie the observed variations in the heat resistance.

In conclusion, it can be stated that the heat treatment of İnegöl meatballs at internal temperature of 85 °C for 4 min is safe against the hazard of *L. monocytogenes* especially when it is at $\leq 10^4$ cfu/g level, and cooking process at an internal temperature of 80 °C for 4 min does not completely eliminate the pathogen in the meatballs containing $\geq 10^2$ cfu/g *L. monocytogenes* but can reduce the levels of pathogen to undetectable numbers. However, it can be concluded that the cooking process carried out at an internal temperature of 63 °C for 30 min is not sufficient for eliminating the high contamination (10^5 and 10^6 cfu/g) of *L. monocytogenes*, and the survival of the pathogen after cooking process indicate the possibility of a public health hazard. Results of this work emphasise the necessity for cooking at an internal temperature of 85 °C for 4 min of the meatballs. These safe practices are especially important, as *L. monocytogenes* have been reported to cause disease, in some cases even when ingested in very low numbers.

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