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# Microbiological Quality of Raw Meat Balls: Produced and Sold in the Eastern of Turkey

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**Abstract:** Raw meat ball is a traditional raw meat product which is consumed particularly in the region of southeast and other parts of Turkey. Since raw meat ball is made from raw ground beef and consumed without cooking, it can be a risk factor for the consumers in terms of foodborne infections and toxications. Thus, its microbiological quality was examined. In ground beef samples, total aerob mesophiles, *Staphylococci* and *Micrococci* spp., *S. aureus*, *Enterobacteriaceae*, coliform, *Enterococci* spp., *Pseudomonas* spp., *B. cereus*, Yeast-Mould, *E. coli*, and *E. coli* 0157 H7 were counted at the average of $3.4x10^4$, $2.2x10^2$, $1.6x10^1$, $1.4x10^3$, $2.0x10^1$, $8.0x10^2$, $9.2x10^3$, $1.2x10^1$, $4.6x10^1$, $3.8x10^3$, and $<2.0x10^2$ cfu/g, respectively. *Salmonella* spp., were determined at the levels of 24.0 % (12 samples). Based on the *Salmonella* spp. and *E. coli* numbers, 24% and 2% samples did not comply with the TFC (Turkish Food Codex) criteria for ground beef, respectively, while all samples did according to the numbers of total aerobic mesophile, *E. coli* O157 H7 and *S. aureus*. In raw meat ball samples total aerob mesophiles, *Staphylococci* and *Micrococci* spp, *S. aureus*, *Enterobacteriaceae*, coliform, *Enterococci* spp., *Pseudomonas* spp., *B. cereus*, Yeast-Mould, *E. coli*, and *E. coli* 0157 H7 were determined at the average of $4.3x10^6$, $1.0x10^5$, $6.3x10^3$, $4.8x10^5$, $1.7x10^4$, $3.1x10^5$, $7.9x10^3$, $1.5x10^1$, $6.7x10^5$, $1.2x10^3$, and $<2.0x10^2$ cfu/g, respectively. *Salmonella* spp., were determined at the levels of 36.0 % (18 samples). Based on the numbers of *Salmonella* spp., *E. coli* and *S. aureus*, 36 %, 8 %, and 28 % samples did not comply with the TFC criteria for raw meat ball, respectively, where as they did according to the numbers of *E. coli* O157 H7. We conclude that consumption of raw meat ball poses a risk of foodborne infections or toxication due to its raw meat content for human health.

**Key words:** Meat balls, raw meat, microbiological quality, hygiene

## Introduction

Raw meat ball is a traditional raw meat product which is consumed particularly in the southeast and other parts of Turkey. Although raw meat ball is produced widely there are no standards established in terms of production methods and technology, food additives and their quantities, ingredients and microbiological quality. Methods that are used during production and ingredients vary depend on the location. However, ground beef meat without fat, Burghol (parboiled cracked wheat), tomatoes or pepper pure, garlic, onion, parsly, olive oil, water, salt, and mixture of herbs-spices are added in general. Traditional raw meat ball is made with naked hands. It requires to be mixed up all the ingredients and to be prepared like a dough by the movements of smashing until required texture is obtained. The microbiological quality of the raw meat and other ingredients, personal hygiene and any contamination during the process will determine the microbiological quality of end product. Studies done on the microbiological quality on ground meat show that ground meat is a good medium for the growth of microorganisms and foodborne infections and toxications can occur due to some bacteria (e.g. *E. coli*, *S. aureus*, *Salmonella* spp. and sulphide reducing anaerobs) (Pivnick *et al*., 1976; Bensink, 1979; Fukushama *et al*., 1987; Ramasastry *et al*., 1999; Davidson *et al*., 2000; Phillips *et al*., 2001) Since raw meat ball is consumed without cooking, it can be a risk factor for the consumers in terms of both foodborne infections and toxications. In this study, the microbiological quality of ground beef meat and raw meat balls were investigated in order to determine its risks for public health.

## Materials and Methods

**Samples:** Fifty samples (about 100 g) of ground beef meat and 50 samples (about 100 g) of raw meat balls made from these ground beef meat were collected from restaurants in Bitlis district (Southeast Turkey). Each sample was placed in an individual sterile plastic bag. Samples were transported to the laboratory immediately after collection in ice-chest and tested upon arrival or stored at 4ºC for no longer than 4 h.

**Microbiological analysis:** To analyze the samples of ground beef meat and raw meat balls, the methods stated in Compendium of Methods for the Examination of Foods (Vanderzant and Splittstoesser, 1992) and Food and Drug Administration (FDA) (Anonymous, 1998)

were used. 10 g of each sample was placed in a sterile stomacher bag containing 90 ml of peptone water. Drop method was used to inoculate agar plates. Aerob mesophiles were determined using Plate Count Agar (Oxoid CM 325), plates incubated at 30°C for 24-48 h. *Enterobacteriaceae* were counted on Violet Red Bile Glucose Agar (Oxoid CM 485) aerobically and incubated at 37°C for 24-48 h. Pink-red colour colonies with precipitation were taken into consideration. Coliforms and *Escherichia coli* (*E. coli*) were determined on Violet Red Bile Lactose Agar (Oxoid CM 107) aerobically and incubated at 37°C for 24-48 h. Pink-red colonies with precipitation were streaked on Endo Agar (Oxoid CM) and incubated at 37°C for 24-48 h. IMViC test was performed on colonies that showed shiny-metallic green to identify *E. coli*. *Enterococci* spp. were counted on Slanetz Bartley Medium (Oxoid CM 377) after incubating aerobically at 37°C for 24-48 h. The red colonies grown on this medium were taken into consideration. *Staphylococci* and *Micrococci* spp. were determined on Baird Parker Agar (Oxoid CM 275) aerobically at 37°C for 24-48h. Typical black colonies with zones around and atypical black colonies were considered as *Staphylococci* spp., small brown-black colonies without zones around were considered as *Micrococci* spp. Colonies that were isolated as *Staphylococci* spp. were inoculated into Brain Heart Infusion Broth (Merck 1.10493), and were incubated at 37°C for 24-48 h. Subsequently coagulase test (Merck 1.3306) was performed to isolate coagulase (+) *Staphylococci* spp. and these isolates were inoculated on DNase Agar (Merck 1.10449) for the identification of *Staphylococcus aureus* (*S. aurues*) and were confirmed by cell morphology, Gram reaction, catalase activity, sensitivity to lysostaphine. *Pseudomonas* spp. were isolated on Pseudomonas Agar (Oxoid CM 559) aerobically at 30°C for 24-48 h. Oxidase (+) colonies were taken into consideration. *Bacillus cereus* (*B. cereus*) was isolated on Cereus Selective Agar (Merck 1.05267) aerobically at 30°C for 24-48 h. Pink-purple, opaque colonies were chosen for further examinations (Gram stain, catalase test, motility test, nitrate reduction, tyrosine decomposition, anaerobic fermentation of glucose, VP reaction, production of acid from mannitol and arabinose). To isolate *Salmonella* spp., 25 g of samples were incubated in 225 ml buffered peptone water (Oxoid CM 509) at 37°C for 24 h. Subsequently 0.1 ml inoculated into Rappaport Vassiliadis Broth (Merck 1. 07700) and were incubated at 43ºC for 24-48 h. Streak plates were prepared on Salmonella Shigella Agar (Merck 1.07667) at 24 and 48 h incubation times and incubated at 37°C for 24-48 h. Pink- red colonies with black centres were inoculated onto Triple Sugar Iron Agar (Merck 1. 03915) and Lysine Iron Agar (Merck 1.11640). Biochemical and serological (Oxoid FT 203) tests were performed for the identification of *Salmonella* spp. For the isolation of anaerobs that capable of reducing sulphide, Tryptose Cycloserin Agar (Merck 1.11972) was inoculated and incubated at 37°C for 24-48 h. Colonies with black zone (or without) were taken into consideration. Rose Bengal Chloramphenicol Agar (Oxoid, CM 0549) was used to isolate Yeast-Mould and incubated anaerobically at 30°C for 4-5 days. To detect the presence of *E. coli* O157 H7 in ground beef samples, a 25 g of sample was pre-enriched with modified novobiocin EC broth (mEC+n, Merck 14582, Berlin, Germany) at 37°C for 24 h. A swap of the enrichment broth was then spread onto selective CT-SMAC (Cefixime-Tellurite Supplement and Sorbitol MacConkey Agar, Oxoid CM 813 and SR 172 E, Basingstoke, UK) and incubated at 42°C for 24-48 h. End of the incubation, colourless, sorbitol negative (-), suspected colonies were streaked onto Fluorocult Violet Red Bile (VRB) (Merck 1.04030, GERMANY) and these plates were incubated at 42°C for 24-48 h. aerobically. Colonies grown on VRB were checked under UV light. The suspected colonies were Gram stained and IMVIC tests were performed. The colonies were then subjected to the agglutination test to determine the serotype of the bacteria using specific antisera to E. coli O157 (Oxoid, 200075, UK) and Dryspot *E. coli* O157 latex agglutination test (Oxoid, UK) for *E. coli* O157 carried out in parallel. Cultures identified as *E. coli* O157 were tested with antisera H7 (Oxoid, 211057, UK) as described by the manufacturer.

**Statistical analysis:** Mann Whitney test was used to analyze the results statistically.

## Results and Discussion

The microbiological quality of ground beef meat and raw meat balls made with these ground beef meat are summarized in Table 1, 2, 3 and 4. In ground beef samples, total aerob mesophiles, *Staphylococci* and *Micrococci* spp., *S. aureus*, *Enterobacteriaceae*, coliform, *Enterococci* spp., *Pseudomonas* spp., *B. cereus*, Yeast-Mould, *E. coli*, and *E. coli* 0157 H7 were counted at the average of $3.4x10^4$, $2.2x10^2$, $1.6x10^1$, $1.4x10^3$, $2.0x10^1$, $8.0x10^2$, $9.2x10^3$, $1.2x10^1$, $4.6x10^1$, $3.8x10^3$, and $<2.0x10^2$ cfu/g, respectively. *Salmonella* spp., were determined at the levels of 24.0 % (12 samples). Based on the *Salmonella* spp. and *E. coli* numbers, 24%, and 2% samples did not comply with the TFC criteria (Anonymous, 2000) for ground beef, respectively, while all samples did according to the numbers of total aerobic mesophile, *E.coli* O157 H7, and *S. aureus*. In raw meat ball samples total aerob mesophiles, *Staphylococci* and *Micrococci* spp, *S. aureus*, *Enterobacteriaceae*, coliform, *Enterococci* spp., *Pseudomonas* spp., *B. cereus*, Yeast-Mould, *E. coli*, and *E. coli* 0157 H7 were determined at the average of $4.3x10^6$, $1.0x10^5$, $6.3x10^3$, $4.8x10^5$, $1.70x10^4$, $3.1x10^5$, $7.9x10^3$, $1.5x10^1$, $6.7x10^5$, $1.2x10^3$, and $<2.0x10^2$

Table 1: The contamination level of analyzed ground meat samples

| | Mean | Min | Max |
|---|---|---|---|
| Total No. of aerob mesophiles | $3.4x10^4$ | $1.0x10^3$ | $9.6x10^5$ |
| *Staph.* and *Micrococci* spp. | $2.2X10^2$ | $2.0x10^2$ | $7.6X10^3$ |
| *S. aureus* | $1.6X10^1$ | $<2.0X10^2$ | $8.0X10^2$ |
| *Enterobacteriacea* | $1.4X10^3$ | $2.0X10^3$ | $4.2X10^4$ |
| Coliform | $2.0X10^1$ | $<2.0X10^2$ | $2.6X10^3$ |
| *Enterococci* spp. | $8.0x10^2$ | $<2.0x10^2$ | $3.6x10^3$ |
| *Pseudomonas* spp. | $9.2x10^3$ | $2.0x10^2$ | $7.6x10^4$ |
| *Bacillus cereus* | $1.2x10^1$ | $<2.0x10^2$ | $6.0x10^2$ |
| Yeast- mould | $3.8x10^3$ | $<2.0x10^2$ | $2.0x10^4$ |
| *E. coli* | $4.6x10^1$ | $<2.0x10^2$ | $3.2x10^3$ |
| *E. coli* 0 157 H7 | $<2.0x10^2$ | $<2.0x10^2$ | $<2.0x10^2$ |

Table 2: The results of analysed raw meat samples

| | Mean | Min | Max |
|---|---|---|---|
| Total No of aerob mesophiles | $4.3x10^6$ | $1.8x10^5$ | $6.2x10^8$ |
| *Staph.* and *Micrococci* spp. | $1.0x10^{6`}$ | $2.0x10^3$ | $9.0x10^6$ |
| *S. aureus* | $6.3x10^3$ | $<2.0x10^2$ | $4.0x10^4$ |
| *Enterobacteriacea* | $4.8x10^4$ | $1.0x10^3$ | $8.2x10^5$ |
| Coliform | $1.7x10^4$ | $2.0x10^2$ | $9.0x10^5$ |
| *Enterococci* spp. | $3.1x10^5$ | $2.0x10^2$ | $7.0x10^5$ |
| *Pseudomonas* spp. | $7.9x10^3$ | $<2.0x10^2$ | $6.0x10^4$ |
| *Bacillus cereus* | $1.5x10^1$ | $<2.0x10^2$ | $3.0x10^3$ |
| Yeast- mould | $6.7x10^5$ | $2.0x10^3$ | $6.0x10^6$ |
| *E. coli* | $1.2x10^3$ | $<2.0x10^2$ | $4.0x10^4$ |
| *E. coli* 0 157 H7 | $<2.0x10^2$ | $<2.0x10^2$ | $<2.0x10^2$ |

cfu/g, respectively. *Salmonella* spp., were determined at the levels of 36.0 % (18 samples). Based on the numbers of *Salmonella* spp., *E. coli* and *S. aureus*, 36%, 8%, and 28% samples did not comply with the TFC criteria (Anonymous, 2000) for raw meat ball, respectively, where as they did according to the numbers of *E. coli* O157 H7. Its microbiological quality depends on the microbiological quality of ingredients such as ground beef meat, tomato, pure, herbs-spices and personal hygiene. Regarding the microbiology of ground beef meat and herb-spices, there are many studies carried out in Turkey (Tekinsen *et al*., 1980; Aksu *et al*., 1997; Agaoglu *et al*., 1999; Erol *et al*., 1999; Uner and Ergun, 1999) and in other countries (Duitschaever *et al*., 1973; Chambers *et al*., 1976; Emswiler *et al*., 1976; Baxter and Holzapfel, 1982; Bhat *et al*., 1987; Kneifel and Berger, 1994; Davidson *et al*., 2000). Westhoff and Feldstein (1976) examined 140 ground beef meat samples and reported coliform, fecal coliform, *E. coli* and total aerob mesophiles at the average of numbers of $2.0x10^2$, $1.0x10^1$, $5.0x10^0$ and $7.9x10^6$ cfu/g, respectively. Duitschaever *et al*. (1973) found total aerob mesophiles and psycrotrophic bacteria in 64% of ground meat samples with the counts more than $>10^6$ cfu/g, *Staphylococci* spp. in 98 % samples with numbers more than $>10^3$ cfu/g. *Enterococci* spp. ranged from 1.0 x $10^1$ to $10^4$ cfu/g. Coliform was found in 95 % samples at the average numbers of $1.0x10^2$ cfu/g. 17% samples contained coagulase (+) *Staphylococci* spp. No *Salmonella* spp. was isolated. Shoup and Oblinger (1976) isolated *Salmonella* spp. and *E. coli* from one sample out of 40. Emswiler *et al*. (1976) reported 4.60 and 4.86 $\log_{10}$ cfu/g of total aerobic mesophiles. Chambers *et al*. (1976) examined 457 ground beef samples and isolated total aerob mesophiles, oxidase (+) psycrophiles and coliform bacteria at the average levels of $10^6$, $10^5$ and $10^2$ cfu/g respectively. Tekinsen *et al*. (1980) examined 20 samples and found that total aerob mesophiles, psychotrophic bacteria, fecal *Streptococci* spp., *Staphylococci* spp., coliform, *E. coli*, bacteria that capable of reducing sulphide and *Clostridium perfringens* were counted at the average levels of $8.4x10^7$, $6.2x10^7$, $1.5x10^5$, $9.6x10^5$, $8.5x10^6$, $4.2x10^6$, $6.7x10^3$ and $3.9x10^2$ cfu/g, respectively. Khalafalla *et al*. (1993) examined 10 ground beef meat samples and total aerob mesophiles, *Enterobacteriaceae* and *Staphylococci* spp. were found at the levels of $10^6$, $10^4$ and $10^3$ cfu/g respectively. Davidson *et al*. (2000) reported coliform and *E. coli* at the level of $1.2x10^4$ and $4.8x10^3$, respectively. *Salmonella* spp. were isolated from six samples out of 47. These results show that the microbial quality of ground beef meat vary depend on the technique used to slaughter animals, contaminations may occur during eviscreation of the internal organs, abattoir hygiene, conditions of storage, personal hygiene. It was reported (Aksu *et al*., 1997; Agaoglu *et al*., 1999; Erol *et al*., 1999, Uner and Ergun, 1999; Temelli and Anar, 2000) that herbs-spices which are used in making raw meat ball such as cumin, black pepper, red pepper in general contained total mesophiles bacteria, *Staphylococci* and *Micrococci* spp, coliform Yeast-Mould and *B. cereus* in the average of $10^4$-$10^7$ cfu/g, $10^2$-$10^3$ cfu/g, $10^2$-$10^4$ cfu/g, $10^3$-$10^5$cfu/g and $10^2$-$10^5$ cfu/g, respectively. The results obtained in this study show that raw meat ball samples had higher numbers of bacteria than ground beef meat samples. The average numbers of total mesophiles, Yeast-mould, *S. aureus*, and *E. coli* were 2 $\log_{10}$ cfu/g, and *Enterobactericeae* were 1 $\log_{10}$ cfu/g higher than ground beef meat used. *Staphylococci* and *Micrococci* spp., coliform and *Enterococci* spp. were 3 $\log_{10}$ cfu/g higher than the average numbers for the same microorganisms in ground beef meat. The level of *B. cereus*, *Pseudomonas* spp., and *E. coli* 0 157 H7 in the ground beef as same as raw meat ball whereas 12.0%, increase was observed in the level of *Salmonella* spp. The differences in the microbial numbers of the ground meat and raw meat balls were statistically significant ($P<0.0001$). These differences may be explained by the microbial quality of ingredients used and personal hygiene. We conclude that consumption of raw meat ball poses a risk of foodborne infections or toxication

Table 3: The distribution of contamination of analysed ground meat samples

| | CFU/g | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $<10^2$ | $10^2$ | $10^3$ | $10^4$ | $10^5$ | $10^6$ | $10^7$ | $10^8$ |
| Total number of aerob mesophiles | - | - | 13 | 17 | 20 | - | - | - |
| *Staph.* and *Micrococci* spp. | - | 39 | 11 | - | - | - | - | - |
| *S. aureus* | 44 | 6 | - | - | - | - | - | - |
| *Enterobacteriacea* | - | - | 34 | 16 | - | - | - | - |
| Coliform | 35 | 12 | 3 | - | - | - | - | - |
| *Entrococci* spp. | 25 | 14 | 11 | - | - | - | - | - |
| *Pseudomonas* spp. | - | 10 | 34 | 6 | - | - | - | - |
| *B. cereus* | 49 | 1 | - | - | - | - | - | - |
| Yeast- mould | 22 | - | 22 | 6 | - | - | - | - |
| *E. coli* | 45 | 3 | 2 | - | - | - | - | - |
| *E. coli* 0 157 H7 | 50 | - | - | - | - | - | - | - |

Cfu/g : Coloni Forming Unit,

Table 4: The distribution of contamination of analysed raw meat samples

| | CFU/g | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $<10^2$ | $10^2$ | $10^3$ | $10^4$ | $10^5$ | $10^6$ | $10^7$ | $10^8$ |
| Total number of aerob mesophiles | - | - | - | - | 10 | 31 | 5 | 4 |
| *Staph.* and *Micrococci* spp. | - | - | 7 | 23 | 11 | 9 | - | - |
| *S. aureus* | 5 | 13 | 18 | 14 | - | - | - | - |
| *Enterobacteriacea* | - | - | 16 | 22 | 12 | - | - | - |
| Coliform | - | 4 | 12 | 28 | 6 | - | - | - |
| *Entrococci* spp. | - | 2 | 14 | 17 | 13 | 4 | - | - |
| *Pseudomonas* spp. | 8 | 14 | 19 | 9 | - | - | - | - |
| *B. cereus* | 29 | 18 | 3 | - | - | - | - | - |
| Yeast- mould | - | - | 7 | 19 | 16 | 8 | - | - |
| *E. coli* | 9 | 14 | 23 | 4 | - | - | - | - |
| *E. coli* 0 157 H7 | 50 | - | - | - | - | - | - | - |

due to its raw meat content for human health. In order to minimize the contamination level of raw meat balls use of best microbial quality ground beef meat and ingredients as well as good personal hygiene are required.

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