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Original Research

The Microbiological, Serological and Parasitological Quality of Cig Kofte (Raw Meatball) and Its Lettuce Marketed in Istanbul

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Abstract

The objective of this study was to investigate microbiological, serological and parasitological quality in cig kofte (raw meatball) and their lettuce consumed in Istanbul. Consequently, a total of 102 cig kofte and 60 lettuce samples were analyzed for total mesophylic aerobic bacteria (TMAB), sulphite-reducing bacteria, coliforms, *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Staphylococcus-Micrococcus*, and *Salmonella* spp. levels. Sulphite-reducing bacteria were not isolated from any of the samples. Salmonella were found positive in 9 of cig kofte and in 5 of lettuce samples. None of the analyzed samples was found to contain pork. Ten of them contained equine, 5 contained beef+mutton, 12 contained beef+poultry and 7 contained equine+poultry, while 68 of the 102 samples were only beef meat. Parasites and their fragments were also observed in some of the cig kofte and lettuce samples (6 insect fragments in cig kofte samples and 2 insect fragments, 5 acarus and mites and 2 *Entamoeba coli* + acarus in lettuce samples). It was concluded that cig kofte and lettuce offered to consumers in Istanbul have low microbiological and parasitological quality. Due to imitation of the products, unfair competition and profit is provided and consequently the safety of the consumer is at risk.

Keywords: cig kofte, lettuce, microbiological, serological, parasitological quality

Introduction

Cig kofte (raw meatball) is a domestic meat product consumed traditionally in Turkey [1, 2]. There is no spesific standard for the composition and ingredients of cig kofte. Generally, raw ground beef and/or lamb meat, bulgur (pounded wheat), salt, tomato paste, onions, garlic, cumin, parsley, black pepper, allspice and various spices are used in the production of cig kofte [3-5]. After mixing the ingredients, it is kneaded by hand and shaped into small portions by hand. Preferably it is wrapped by a small piece of lettuce with lemon juice and should be consumed within a few hours of production [6].

The hygienic quality of raw materials used in the production of cig kofte is very important. It has been reported by numerous researchers in Turkey that ground meat and

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spices were contaminated by severe pathogenic bacteria such as *Staphylococcus aureus*, coliform bacteria, *Escherichia coli, Enterococci* and *Bacillus cereus* [7-13].

Lettuce consumed with cig kofte also harbour parasites and pathogenic bacteria such as *E. coli, Salmonella* spp. and *L. monocytogenes* [14-16].

Consumption of cig kofte produced from contaminated pork meat was responsible for a large outbreak of trichinellozis that sickened more than 600 people in Izmir, Turkey, between December 2003 and January 2004 [17].

In this study, the microbiological, serological and parasitological properties of ready-to-eat cig kofte and the microbiological and parasitologycal properties of lettuce sold in Istanbul have been investigated regarding consumer safety.

Materials and Methods

Sampling of Foods

The samples were collected in autumn, winter, and spring from different parts of Istanbul, Turkey. All samples were carried to the laboratory within sterilized glass jars kept in ice chests and examined on the same day. A total of 102 cig kofte and 60 lettuce samples were collected from different markets (38%) and street sellers (62%). The samples were analyzed immediately for microbiological, serological and parasitological parameters.

Microbiological Analyses

Portions of cig kofte and lettuce (25g) were transferred to a sterile stomacher bag with 225 mL of 0.1% peptone water (Oxoid CM 9, UK) and homogenized for 2 min. (Labblender 400, Model BA 6021, Steward Lab., London, UK). Serial decimal dilutions were prepared using the same diluents up to 10^{-7}. Total aerobic bacteria counts were detected using plate count agar (Oxoid CM463, UK) incubated at 30°C for 48 h [18, 19].

S. aureus was defined on Baird–Parker agar (Oxoid CM0275) supplemented with egg yolk–tellurite emulsion (Oxoid SR0054). Spread plates were incubated at 35°C for 24h. Colonies with typical S.aureus morphology were examined microscopically following Gram staining, and tested for catalase and coagulase activity, and confirmed with DNase Agar (Oxoid CM0321) incubated at 35°C for 18-24 h [18, 19].

Coliform was examined in Violet Red Bile Agar (Oxoid, CM0107) by using pour plates with overlay added before incubation, and incubated at 35°C for 24h [19]. *E. coli* was determined according to ISO 16649-2 [20] procedure in TBX Agar (Oxoid, CM0945), pour plates incubated at 44°C for 24h. Polymyxin–pyruvate–egg yolk–mannitol–bromothymol blue agar (Oxoid CM617) with 50,000 IU of polymyxin per litre and egg yolk emulsion (Oxoid SR47) was used for *B. cereus* counts incubating at 30°C for 24–48h and all other further analyzes were performed according to Holbrook and Anderson [21].

Yeast and mould were defined on Yeast Extract Glucose Chloramphenicol Agar (Merck 1.16000). Spread plates were incubated at 25°C for 3-5 days [19]. Sulphite-reducing bacteria were determined in Perfringens Selective Agar (Merck, 1.10235, Darmstadt, Germany). Roll tube method was used for incubation at 35°C for 24h [19].

Isolation of *Salmonella* spp. was carried out in four steps. 225 mL Buffered Peptone Water (Oxoid, CM0509) was added to 25 g raw meat ball sample and incubated at 35-37°C for 16-20 h for pre-enrichment step, 0.1 and 1 mL of the homogenate was transferred to Rappaport Vassiliadis medium (Oxoid, CM0669) and Tetrathionate Broth (Oxoid, CM0671) for selective enrichment with an incubation period of 42 and 43°C for 24 h, respectively. After incubation a loopful from each tube was streaked on Bismuth Sulfite Agar (Oxoid, CM0201), Xylose Lysine Desoxycholate Agar (Oxoid, CM0469) and Hectoen Enteric Agar (Oxoid, CM0419), and incubated for 20-24 h at 35°C. Typical colonies were checked and selected for growing on Nutrient Agar (Oxoid, CM0003) at 35°C for 18-24 h and then were identified by Triple Sugar Iron Agar (Oxoid, CM0277), Lysine Iron Agar (Oxoid, CM0381) fermentation tests, urease test (Oxoid, CM0071), Voges proskeuer, indol, O-, Vi- and H-antigen tests (Murex Salmonella Polyvalent Agglutinating Sera) [18].

Determination of Animal Species with Agar Gel Immunodiffusion Test (AGID)

Ten mL of 1.0% agar solution (Oxoid, UK) in 0.85% saline was poured into a Petri dish 10 cm in diameter. Several wells were prepared in the agar gel at a distance of 3 mm between the wells. The petri dishes were stored in a refrigerator until use. Mince meat extractions were prepared using 25 g samples and 75 mL normal saline solution. They were stomached for 5-10 minutes and left to stand a minimum of 90 minutes at room temperature. After decanting liquid and filtering through Whatman No. 42 filter paper, the extracts were used immediately. Each well in the gel was filled with the sample and serum to be tested. Undiluted anti-horse, pork, beef, sheep, and chicken species sera were added into the wells. Petri dishes were incubated for 24 to 48 h. at room temperature. The reactant bands appeared white on a grey surface [22].

Determination of Parasites

Parasites and their fragments were detected according to sedimentation method reported in MAFF [23]. Trichinella spp and larvaes were identified by trichinoscope method [24].

Results and Discussion

Microbiological Analysis

The aim of this study was to determine the elements causing quality destruction in cig kofte and lettuce consumed with it, and to establish the risks for public health by

Table 1. The results of microbiological analysis of cig kofte samples (n=102).

Microorganisms	Min (cfu/g)	Max (cfu/g)	Average (cfu/g)	n^p	Rate of positive samples (%)
Total mesophylic aerobic bacteria counts (TMAB)	1×10^4	6.4×10^7	6.5×10^6	102	100
Sulphite-reducing bacteria	-	-	-	-	-
Yeast and mould	1×10^3	7.1×10^6	4.7×10^5	102	100
Coliforms	2×10^2	8.5×10^5	3.2×10^4	90	88.23
E.coli	1×10^1	4.3×10^3	3.8×10^2	40	39.21
B.cereus	1×10^2	4.5×10^4	6.3×10^3	30	29.41
S.aureus	1×10^2	2×10^4	3.4×10^3	45	44.11
Staphylococcus-Micrococcus	1×10^2	1×10^6	3.1×10^4	102	100
Salmonella spp.	-	-	-	9	8.82

n: number of analysed samples
n^p: number of positive analysed samples

Table 2. The Results of microbiological analysis of lettuce samples (n=60).

Microorganisms	Min (cfu/g)	Max (cfu/g)	Average (cfu/g)	n^p	Rate of positive samples (%)
Total mesophylic aerobic bacteria counts (TMAB)	1×10^4	2.5×10^7	3.6×10^6	60	100
Sulphite-reducing bacteria	-	-	-	-	-
Coliforms	2.5×10^2	1.4×10^6	3.2×10^5	58	96.66
E.coli	1×10^1	8×10^3	1.4×10^3	28	46.66
Salmonella spp.	-	-	-	5	8.33

n: number of analysed samples
n^p: number of positive analysed samples

evaluating the microbiological, serological and some parasitological properties of ready-to-eat cig kofte sold at different districts of Istanbul. It was consequently aimed at achieving customer safety by avoiding product imitation and adulteration and also unfair competition and income.

The results of microbiological analysis of 102 cig kofte samples are given in Table 1. Total mesophilic aerobic bacteria count (TMAB) varied between $1x10^4$-$6.4x10^7$ cfu/g and average of TMAB was determined as $6.5x10^6$ cfu/g. These results are in accordance with those reported by Arslan et al. [25], Kuplulu et al. [26], Sagun et al. [11] and Daglioglu et al. [6]. Goktan and Tuncel [27] reported that TMAB levels in minced meat used for cig kofte production were 10^5cfu/g and this level was stable for the final product. However, Erol et al. [7] stated that the TMAB count in ground meat used for cig kofte production was 10^5 cfu/g, but increased up to 10^7 cfu/g after 24 hours. Meat from healthy animals might also be exposed to various degrees of bacterial contamination during production process of cig kofte, like minced meat storage, packaging and marketing [24].

Microbiological contamination occurs commonly during minced meat preparation. A study has revealed that minced meat prepared at butcheries contains 12.5, 20 and 2.5 times more TMAB, enterobacter and staphylococcus, respectively, than minced meat prepared at home [28].

Sulfit-reducing bacteria were not found in any of the cig kofte samples obtained from restaurants, markets and butchers in Istanbul.

While coliform bacteria count was $3.2x10^4$ cfu/g, in 90 (88.23%) samples, *E. coli* count was $3.8x10^4$ cfu/g, in samples (39.21%). These results are in accordance with those of Arslan et al. [25] and Kuplulu et al. [26], who reported that the coliform levels in cig kofte samples were 10^4 cfu/g. However, Sagun et al. [11] reported that 5.2×10^3 cfu/g coliforms in cig kofte samples. The reason of different results of coliforms bacteria in the cig kofte samples is because of the varied usage of raw materials and ingredients. Moreover, Daglıoglu et al. [6] reported that *E. coli O157:H7* was contained in all 30 cig kofte samples marketed in Istanbul.

Table 3. Determination of animal species in cig kofte samples (n=102).

Animal species	Number of samples	Percentage of samples (%)
Beef	68	66.66
Beef + Mutton	5	4.90
Beef + Poultry	12	11.76
Equine + Poultry	7	7.84
Equine	10	9.80
Pork	-	-

In this study, *Salmonella* spp. were isolated only in 9 (8.82) of the 102 samples. In a study conducted by Kuplulu et al. [26] *Salmonella* was not isolated from any of the 50 cig kofte samples analyzed.

Gokmen and Alisarli [8] have investigated various pathogen bacteria in 100 minced beef and 100 minced lamb samples obtained from retail markets and butcheries in Van, Turkey. They observed *Salmonella* spp, in 3% and 4% of the minced meat samples obtained from beef and lamb, respectively. Although Erol [29] determined the incidence of *Salmonella* in ground meat to be 3.3%, while Tekinsen et al. [13] reported that *Salmonella* could not be isolated from ground meat samples. Uzunlu and Yildirim [30] observed no change in *Salmonella Enteritis* count in the cig kofte samples inoculated with 10^3 cfu/g of the bacterium and stored at 4°C for 24 hours. Goktan and Tuncel [27] reported that *Salmonella Typhimurium* count decreased from $2x10^2$ cfu/g to $0.8x10^2$ cfu/g after 48 hours of storage.

In the present study the mean level of the mould and yeast was $4.7x10^5$ cfu/g in all cig kofte samples studied. Our results are concordant to those reported by Kuplulu et al. [26] and Daglioglu et al. [6] who detected 10^4 and 10^3 cfu/g of the mould and yeast, in cig kofte, respectively. The spices that are added in cig kofte may raise the total count of the yeast and mould because of high rate of mentioned microorganisms that they include naturally. Erol et al. [31] and Tekinsen and Sarigol [12] reported the total amount of the spices that they have analyzed as 10^3 and 10^3-10^4 cfu/g, respectively.

In this study, the mean level of *Bacillus cereus* was determined to be $6.3x10^3$ cfu/g in 30 (29.41%) of the 102 cig kofte samples. Erol et al. [31] and Tekinsen and Sarigol [12] found *Bacillus cereus* 10^2 to 10^5 cfu/g in various types of spices. Also Pehlivanlar and Sireli [32] reported that *B. cereus* was found in 22 of 50 cig kofte samples sold in Ankara and mean contamination level was 2.55 $\log_{10}$ cfu/g (3.54×10^2 cfu/g) in positive samples. Again in the same study, survival ability of *B. cereus* and its enterotoxin production ability were determined in experimentally prepared raw meatballs inoculated with the enterotoxigenic *B. cereus* NCTC-11145 at the levels of 10^6 cfu/g and toxin production was determined at 8, 12 and 24 hours at 25ºC. Kuplulu et al. [26] reported that the numbers of *B. cereus* was determined as 10^2-10^4 cfu/g in 46% of the samples.

In this study mean *Staphylococcus-Micrococcus* count was $3.1x10^4$ cfu/g in all cig kofte samples while the level of the *Staphylococcus aureus* in 45 (44.11%) of cig kofte samples was $3.4x10^3$ cfu/g. Daglioglu et al. [6] reported that the mean *Staphylococcus aureus* count in cig kofte samples collected from Istanbul was $1.5x10^3$ cfu/g. Arslan et al. [25] determined a level of coagulase positive *Staphylococci* as 10^3-10^5 cfu/g. Kuplulu et al. [26] reported that the mean level of coagulase positive *Staphylococcus aureus* to be 10^2-10^3 cfu/g in 18% of the samples. Erol et al. [7] determined that *Staphylococcus aureus*, inoculated in cig kofte as a level of 10^3-10^5 cfu/g, did not grow. Sagun et al. [33] reported that cig kofte contaminated with *Staphylococcus aureus* at the level of 10^5 cfu/g produced enterotoxin while stored at room temperature (21-23ºC) for 24 h and 30ºC for 12h.

None of the lettuce samples obtained with cig kofte from restaurants and markets in Istanbul was found to contain Sulfit-reducing bacteria while *Salmonella* was isolated only in 5 of 60 lettuce samples (8.33%). Total mesophilic aerobic bacteria count (TMAB) varied between $1.0x10^4$ cfu/g and $2.5x10^7$ cfu/g and mean level was $3.6x10^6$ cfu/g in all samples of the lettuce. While mean level of coliform bacteria was $3.2x10^5$ cfu/g in 58 (96.66%) of samples, *E. coli* was detected in 28 samples (46.66%) to be $1.4x10^3$ cfu/g. *Listeria monocytogenes* was grown on shredded and ready-to-eat iceberg lettuce. Aycicek et al. [34] showed that fresh salad vegetables might contain pathogenic microorganisms and represent a risk for consumers regarding foodborne disease. In another study, total mesohilic aerobic

Table 4. The results of parasitological analysis of cig kofte and lettuce samples.

Parasites	Cig Kofte (n=102)	Positive % of cig kofte samples	Lettuce (n=60)	Positive % of lettuce samples
Fragments of insects	6	5.88	2	3.33
Acarus & Mites	-	-	5	8.33
Amoeba & Ciliates	-	-	2 (*Entamoeba coli*)	3.33
Coccidian parasites (*Sarcocystis* spp.)	-	-	-	-
Food-Borne Nematode (*Trichinella* spp.)	-	-	-	-
Cestodes (Parasite cysts)	-	-	-	-

microorganisms were reported greater than 10^7 cfu/g in 16 of 30 lettuce samples [35].

Parasitological Analysis

Insect fragments were observed in 6 (5.88%) of 102 cig kofte samples. No *Trichinella* spp. and *Sarcocystis* spp. were detected in the analyzed samples. Also no parasite cyst was observed. However, during analyzes, fragments of plastic were found in 2 samples (1.96%).

Insect fragments and acarus were detected in 2 (3.33%) and 5 (8.33%) of 60 lettuce samples, respectively. Furthermore, *Entamoeba coli* were observed together with acarus in 2 (3.33%) of the analyzed samples.

In accordance with our results, De Santana et al. [36] decleared the presence of *Endolimax nana/Endolimax* sp., *Entamoeba histolytica/Entamoeba dispar* and *Entamoeba coli/Entamoeba* sp. in 4, 6 and 2 lettuce samples as 6.7%, 10% and 3.3%, respectively. Paula et al. [35] reported that cysts of *Entamoeba coli* were found in 3 of 30 samples of lettuces collected from the self service restaurants of Nitereoi.

Serological Analysis

None of the cig kofte samples obtained from restaurants, markets and butchers in Istanbul was found to contain pork. Ten of the 102 analyzed cig kofte samples contained equine, 5 contained beef+mutton, 12 contained beef+poultry and 7 contained equine+poultry, while 68 of the 102 samples contained only beef meat. Baskaya et al. [37] and Yildiz et al. [38] have not found any traces of equine and pork meats in ready-to-sell raw minced meat and meatballs, in Istanbul. Cetin et al. [39] have studied 223 raw and heat processed meat products and detected that 83 samples (36.8%) contained meat products, which was not reported on their labels. Of these, 4 (1.8%) are reported as pork, and 78 (35%) as poultry meat.

Conclusions

It has been observed that microbiological quality of ready-to-eat cig kofte and lettuces consumed with cig kofte in Istanbul is very low. The absence of pork meat can be considered a favorable finding, but the presence of parasites in lettuces could be important for human health. Moreover, the results of the analyzes show that meats of animals are not recorded on the labels. This is evaluated as a potential risk factor for public health and for consumer rights, and it is also considered an unfair income source. In order to produce high quality and healthy minced meats and other ingredients in compliance with Turkish Food Codex, it is necessary to obey the ethical values and hygienic rules while processing. On the other hand, these results show the need of implementation of the legislation of cig kofte in TFC. Also, slaughters should be healthy and controlled; secondary and cross contaminations should be prevented, continuity the of the cold chain at transfer and storage should be guaranteed; personnel education and consumer awareness should be targeted.

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