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SIVAS KOFTE AND EXAMINATION OF MICROBIOLOGICAL QUALITY

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Abstract: The objective of this study was to examine traditional meat product of the Sivas province, the Sivas kofte with regards to its microbiological quality. The kofte samples sold commercially were examined according to their microbiological qualities (150 pieces cooked kofte samples taken from the most popular 5 restaurants). The samples were analyzed in terms of total mesophilic aerobic bacteria, Enterobacteria, *E. coli,* coagulase positive *S. aureus*, *Salmonella* spp. and psychrophilic bacteria. Ready to serve samples of Sivas kofte were examined and the following results were obtained for total mesophilic aerobic bacteria, Enterobacteria, coagulase positive *S. aureus*, psychrophilic bacteria, 2.7- 4.9 $\log_{10}$ cfu/g, <10 - 2.1 $\log_{10}$ cfu /g, <10 - 1.9 $\log_{10}$ cfu /g, 1.6- 3.8 $\log_{10}$ cfu /g, respectively. *E. coli* and *Salmonella* spp were not determined in any of the samples. As a result, the ready to consume Sivas kofte samples were found to be in accordance with the Turkish Food Codex Cominiquate Microbiological Criteria despite differences in the microbiological quality of the locations in Sivas.

Key words: Food hygiene, Microbiological quality, Sivas kofte.

Introduction

Nowadays, the changes in individual's consumption habits and advances in food technology have caused an increase in the demand for different style convenience and semi-processed convenience foods. Meatball is one of the most preferred foods due to the ease of preparation (*Anar, 2010*). The meat product prepared by using fresh mince and by shaping the meat dough, which is mostly consumed after grilling is known as a meatball (*TSE 1992*).

Meatballs prepared according to traditions of different regions (Inegol, Akcaabat, Sivas etc.) according to the content of meat dough has an important place in Turkish cuisine. Sivas kofte is one of these kinds and it is a meat product which has a geographical patent and is consumed by the local community. Sivas kofte which is a special product both in terms of its preparation and form of consumption is identified with the city of Sivas. Sivas kofte is produced in three phases of raw material, preparation and cooking. In order to get the special taste of the meatball the meat should be obtained from beef cattle or sheep which raised and bred in plateaus of the Sivas region and fed with clover, *vicia sativa* and marjoram. The rib, leg, and shoulder meat of beef cattle of these plateaus at the minimum age of two years along with leg of sheep are used as raw material. 20 g of salt is added for each kg of the mixture prepared and it is ground in mincing machine. No other material other than salt is used during the preparation of the mixture. The salt used is natural unprocessed salt produced in Tuzlagolu village, Zara district, Sivas. The ground meat mixture is left to rest for 12 hours. The meat is then again ground in the mincing machine with a moderate aperture. The mixture is sliced in to 25 g slices and an oval shape is given to the meat by hand. The meatballs prepared are grilled over an intense char coal fire by turning upside down in short intervals to cook both sides (*RG, 2010*).

Meatball is prepared by mince which is a quite suitable environment for microbial growth. The quality of mince and other additives used in preparation of meatballs determines the quality of meat products like meatballs (*Başkaya et al., 2004*). *E. coli*, *S. aureus*, *Cl. perfringens*, *B. cereus*, *Listeria* spp. and *Salmonella* spp. Together with other pathogens have negative effects on product quality and public health. The reasons of microbial growth in such food are high loads of microorganisms in food raw materials, inadequate thermal treatment, contaminant material, preservation in unsuitable environment, inadequate processing hygiene, cross contamination and unconscious personnel (*Gülmez et al., 2005*). Studies done on microbiological quality on ground meat show that ground meat is a good medium for the growth of microorganism like *S. aureus*, *Salmonella* etc (*Davidson et al., 2000*; *Philips et al., 2001*).

Since meatballs are sold as raw, they can spoil easily and contain some pathogens that pose threats to human health. The studies performed in Turkey showed that the microbial qualities of the meatballs are low and that some contain pathogen mechanisms (*Erol, 2007*).

This study was prepared to introduce Sivas kofte which is a traditional meat product of Sivas region, to determine its microbial quality and to protect public health.

Materials and Methods

The meatball samples examined in this study were taken from five restaurants selling the highest amount of meatballs in Sivas city centre. Sampling was done for 5 days in the restaurants and two groups of meatball samples were taken in each of the sampling days. A group of meatball sample is comprised of three meatballs. A total of 30 meatballs were sampled from each restaurant (6 pieces/day). Thus, a total of 150 grilled meat samples were sampled randomly just prior to the service. The samples were brought under cold chain and analyses were initiated on the same day.

10 g of meatball samples were taken into plastic bags under aseptic conditions and 90 ml % 0.1 water with peptone was added and stirred for 3 minutes after which serial distillations were prepared (ICMFS 1982).

Plate Count Agar (Oxoid CM325) medium was used for total mesophilic aerobic bacteria count. Petri plates were incubated at 35°C for 48-72 hours (*ICMFS, 1982*).

For Enterobacteria count Violet Red Bile Glucose Agar (VRB, Oxoid CM485) medium was used. Petri plates were incubated at 37±1 ºC for 18-24 hours (*ICMFS, 1982*).

25 g of sample was homogenized within 225 ml Maximum Recovery Diluent for *E.coli* determination. Hence, 10^{-1} dilution was prepared and was placed in a 0.5 ml Chromocult Tryptone Bile X-Glucuronide Medium (TBX) (CM945) which had been prepared before in accordance with the agar diffusion method. Then they were hold at 30±1°C for 4±1 hours and incubated at 44±1°C for 18±2 hours. After incubation blue-green coloured colonies in the medium were evaluated as *E. coli*. Since chromogenic medium was utilized confirmation was not done. As a positive control *E. coli* ATCC 25922 strain was used (*ICMFS, 1982*).

For psychrophilic bacteria counting Plate Count Agar (PCA, Oxoid CM325) medium was used. The plates were incubated at 7°C for 10 days (*ICMFS 1982*).

Coagulase positive *S. aureus* counting: Baird Parker (BP) agar (Oxoid CM275) was used for *Staphylococcus* counting. Petri plates were incubated at 37±1°C for 30 hours. After incubation the number of colonies which had a typical black coloured view surrounded by a light coloured area and atypical colonies were determined. Afterwards, five of these colonies were taken and coagulase test was employed. The number of the colonies having a positive coagulase test result was multiplied by suspicious colonies and divided into 5 so that the number of positive *S. aureus* was obtained (*ICMFS, 1982*).

For the isolation of *Salmonella* 25 g of sample was homogenized in 225 ml buffered water with pepton (T.P.S) during pre-enrichment stage and incubated at 37°C for 24 hours. During the selective enrichment stage, however, they were transferred into Rappaport Vassiliadis (R.V.) broth (Oxoid CM669) through 0.1 ml

TPS and incubated at 42°C for 24-48 hours. Then they were put into Brilliant Green Agar (B.G.A.) (Oxoid CM263) via circular loop and incubated at 37°C for 20-24 hours. The pink-reddish coloured colonies surrounded by bright red area in B.G.A. were evaluated as suspicious *Salmonella* spp. From these colonies some were put into Triple Sugar Iron Agar (T.S.I.A.) (Oxoid CM277) and Lysine Iron Agar (L.I.A.)(Oxoid, CM381) slant agar and incubated at 37°C for 24 hours. At the end of the incubation positivity evaluation of tubes was done according to the change in colour in T.S.I.A and L.I.A. Salmonella antiserum (Salmonella O Poly A-1 and Vi-Difco 2264-47-2) was used to test suspicious *Salmonella* spp. And the ones with positive agglutination formation were evaluated (*ICMFS, 1982*).

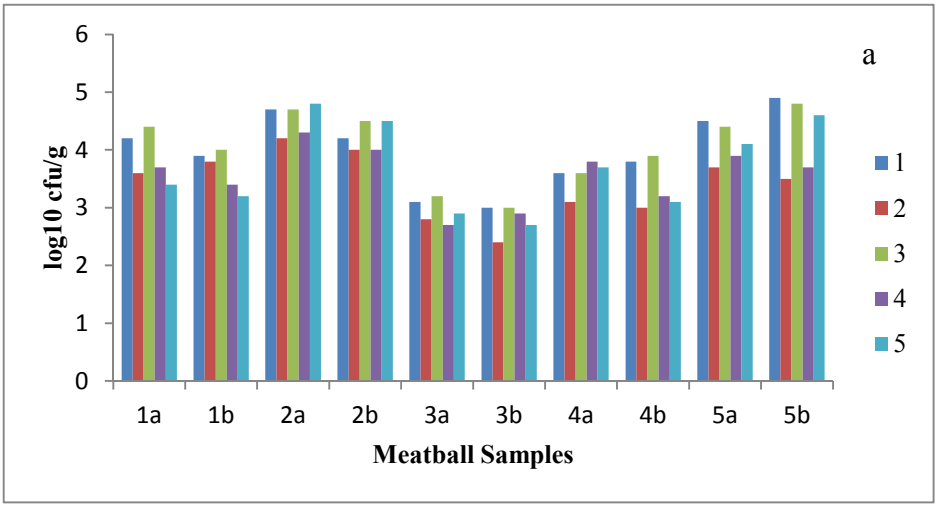
Results and Discussion

The number of total mesophilic aerobic bacteria, *Enterobacteria*, coagulase positive *S. aureus* and psychrophilic bacteria in 150 Sivas kofte obtained from five restaurants selling the highest amount of meatballs in Sivas city centre were found as 2.7-4.9 $\log_{10}$ cfu/g, <10 - 2.1 $\log_{10}$ cfu/g, <10 - 1.9 $\log_{10}$ cfu/g, and 1.6- 3.8 $\log_{10}$ cfu/g, respectively. No *E.coli* and *Salmonella* spp. Was determined in any of the meatball samples. The minimum and maximum values belonging to the meatball samples were given in Table 1. Microbiological analysis findings for the samples were given by Figure 1.

Table 1. The minimum and maximum values belonging to the meatball samples ($\log_{10}$ cfu/g)

Microorganism	No. of samples	Minimum	Maximum	No. of postive samples* (%)
Total mesophilic aerobic bacteria	150	2.7	4.9	150 (100)
Enterobacteria	150	<10	2.1	102 (68)
Coagulase positive *S. aureus*	150	<10	1.9	57 (38)
Psychrophilic bacteria	150	1.6	3.8	150 (100)

* The numbers determined over the determination limit are accepted as positive.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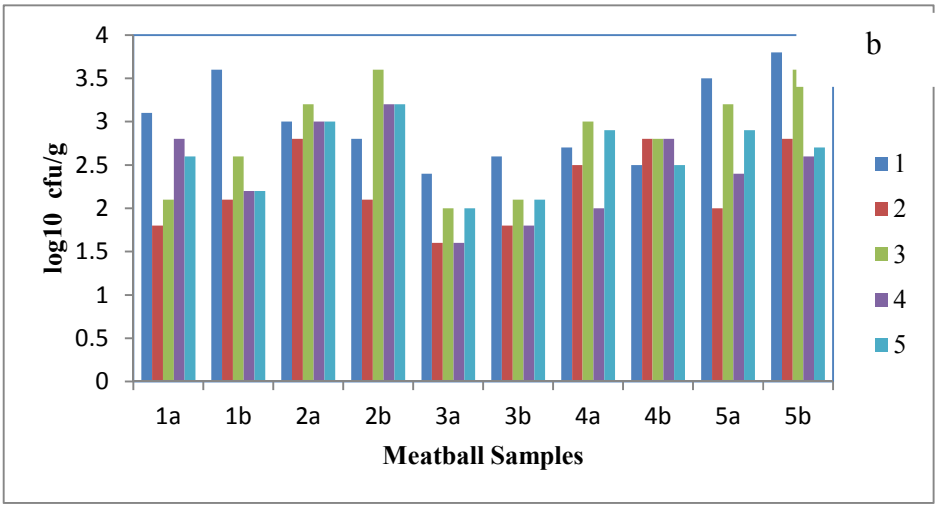
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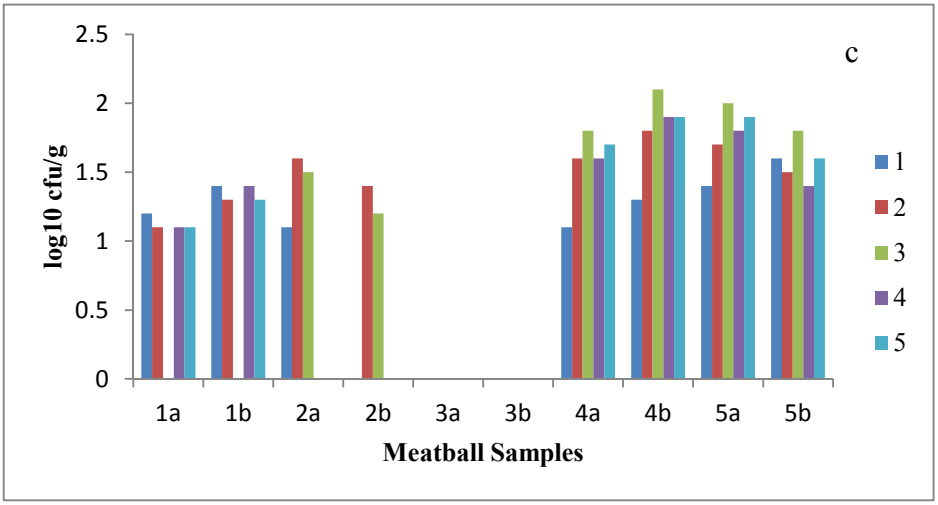

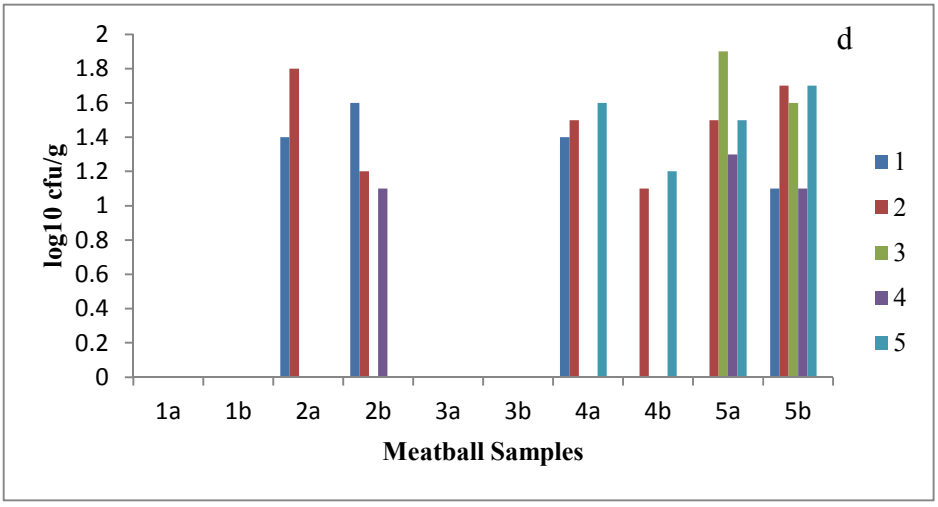

Figure 1. a: Number of total mesophilic aerobic bacteria, b: Number of Psychrophilic bacteria, c: Number of Enterobacteria, d: Number of coagulase positive *S. aureus*, 1a: First group meatballs taken from the first restaurant, 1b: Second group meatballs taken from the first restaurant, 2a: Second group meatballs taken from the first restaurant, 2b: Second group meatballs taken from the second restaurant, 3a: First group meatballs taken from the third restaurant, 3b: Second group meatballs taken from the third restaurant, 4a: First group meatballs taken from the fourth restaurant, 4b: Second group meatballs taken from the fourth restaurant, 5a: First group meatballs taken from the fifth restaurant, 5b: Second group meatballs taken from the fifth restaurant.

This study was performed to investigate the microbiological quality of Sivas kofte which is among conventional foods. There have been some researches regarding the microbiological qualities of raw and cooked meatballs and the researchers reported low qualities (*Sarımehmetoğlu et al., 1998, Soyutemiz, 1999, Yıldız et al., 2004; Kök et al., 2007*). During the handling, packaging, or serving of cooked products, some low level of contamination invariably occurs on the surface of the products from equipment and food handlers (*Johnston* and *Tompkin*, 1992).

It was determined that the number of total mesophilic aerobic bacteria determined in the meatball samples ranges between 2.7 and 4.9 $\log_{10}$ cfu/g. It was observed that this finding is lower than those reported by *Kıvanç* and *Kunduhoğlu* (1996) ($4.35x10^7$ cfu/g in Eskişehir) and *Yıldız et al.* (2004) ($5.6x10^5$ cfu/g in İstanbul) whereas it is closer to those found in a study performed by *Hampikyan et al.,* (2008) in İstanbul ($1.6x10^2$-$3.8x10^5$ cfu/g).

In a study performed in Ankara by *Aycicek et al.* (2005) in 17 (11.8 %) of 144 meatball samples coagulase positive *S. aureus* was determined at a level of 3.7-4.1 $\log_{10}$ cfu/g. Moreover, *Hampikyan et al.* (2008) reported coagulase positive *S. aureus* in 4 (20 %) of the 20 meatball samples between $<10^2$-$2.6x10^4$ cfu/g and *Gülmez et al.* (2005) determined that 4 (10 %) grilled meatball samples were contaminated by *S. aureus* over 10^2 cfu/g.

As it is well known, food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination, via manual contact or through respiratory secretions (*Argudin et al*. 2010). Some beef patties (14.8%) showed absence of *S. aureus*. The presence of small number of *S. aureus* is not uncommon (*Adams* and *Moss*, 2000). Human contact with cooked food invariably adds *S. aureus* at levels 10^1 or 10^2 to many sample units (*Surkiewicz*, 1973). Such levels are harmless but offer sufficient inoculum for growth (*Johnston* and *Tompkin*, 1992). The detection of *S. aureus* in beef patties, could have resulted result from food handlers, animal or environmental sources (*Lancette* and *Tatini*, 1992). In processed foods, in which *S. aureus* is destroyed by processing, its presence usually indicates contamination from the skin, mouth or nose of food handlers. An average prevalence of 19.8% *S. aureus* was found in 10 ready - to - eat consumer food (*Adesiyun et al*. 1995). Also, in this study, it was observed that Sivas kofte sample was in compliance with the limit values stated in the Microbiological Criteria of Turkish Food Codex (*RG, 2011*). Determining *S. aureus* in cooked products reveals both the inadequacy of thermal treatment applied onto the product and the necessity for staff hygiene. *S. aureus* contamination in cooked products is usually due to by employees' hands.

It was determined that the number of psychrophilic bacteria which determines the shelf life of the product changes between 1.6 and 3.8 $\log_{10}$ cfu/g. *Soyutemiz* (1999) determined the number of psychrophilic bacteria as $2.73x10^8$ cfu/g whereas *Kıvanç* and *Kunduhoğlu* (1996) found it as $3.88x10^5$ cfu/g for

cooked meatballs. It can be seen that the findings obtained in this study are lower than those reported earlier.

No *E. coli* was found in any of the Sivas kofte samples. *Hampikyan et al.,* (2008) determined coliform group microorganisms in 8 (40 %) of the 20 meatball samples ranging from 10^1 to 10^4 cfu/g. They found that 3 (15 %) samples contained *E.coli* at levels changing between 10^1-10^3 cfu/g. *Soyutemiz* (1999) determined an average number of coliform bacteria between 10^4 and 10^5 cfu/g. E coli was found in 33.3 % of these meatball samples. In their study, *Yıldız et al.,* (2004) found the number of coliform bacteria as 5.2 x10^3. *E.coli* was determined in 32 % (24/75) of the samples.

Davidson et al. (2000) reported coliform and *E. coli* at the level of 1.2x10^4 and 4.8x10^3, respectively. These results show that the microbial quality of ground meat vary depend on the technique used to slaughter animalsi contaminations may accur during evisceration of the internal organs, conditions of storage, personal hygiene.

According to Turkish Food Codex, *Salmonella* should not be detected in 25 g of the food samples. Improper preparation and handling of foods at food service establishments are primary factors in *Salmonella* outbreaks (*Jay*, 1992). In recent studies, authors reported data related to the contamination of minced meats with *Salmonella* and other foodborne pathogen bacteria in Turkey (*Soyutemiz,* 1999; *Gülmez et al.,* 2005, *Hampikyan et al.,* 2008) and other countries (*Parisi et al*., 2010; *Wojcik et al.,*2010).No *Salmonella* spp. was determined in any of the Sivas kofte samples. This finding is in compliance with those reported by *Soyutemiz* (1999), *Gülmez et al.* (2005), *Hampikyan et al.* (2008). This result is considered as positive for public health.

Outbreaks of human salmonellosis, resulting from ingestion of animal originated foods contaminated with S. Typhimurium, have been reported in many countries (*Ethelberg et al*., (2008); *Mank et al*., (2010)).

Conclusion

In conclusion, though it differs according to the place where they were taken from, the microbiological quality of Sivas kofte in restaurants of Sivas were found suitable according to the Microbiological Criteria Regulation of Turkish Food Codex.

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"Sivas kofte" i ispitivanje mikrobiološkog kvaliteta

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Rezime

Cilj ovog istraživanja je bio da se ispita tradicionalni proizvod od mesa iz pokrajine Sivas – "sivas kofte" u pogledu mikrobiološkog kvaliteta. Uzorci proizvoda koji se prodaje na tržištu su ispitani sa stanovišta mikrobiološkog kvaliteta (150 komada kuvanih uzoraka proizvoda – kofte, koji su uzeti iz 5 najpopularnijih restorana). Uzorci su analizirani u pogledu ukupnih mezofilnih aerobnih bakterija, enterobakterija, *E. coli*, koagulaza pozitivnih *S. aureus*, *Salmonella* spp. i psihofrilnih bakterija. Uzorci proizvoda spremnog za konzumiranje/serviranje su ispitani i dobijeni su sledeći rezultati: ukupan broj mezofilnih aerobnih bakterija, enterobakterija, koagulaza pozitivnih *S. aureus*, i psihofrilnih bakterija - 2.7-4.9 $\log_{10}$ cfu/g, <10 - 2.1 $\log_{10}$ cfu /g, <10 - 1.9 $\log_{10}$ cfu /g, 1.6- 3.8 $\log_{10}$ cfu /g, respektivno. .*E. coli* i *Salmonella* spp. nisu utvrđene ni u jednom uzorku. Kao rezultat ispitivanja, utvrđeno je da su „sivas kofte" – spremne za konzumiranje, odgovaraju turskom standardu odnosno Pravilniku koji se odnosi na mikrobiološki kvalitet - Turkish Food Codex Cominiquate Microbiological Criteria, uprkos razlikama u mikrobiološkom kvalitetu na različitim lokacijama u pokrajini Sivas.

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