

Kars'da Satışa Sunulan Emülsifiye Tipi Et Ürünlerinin Mikrobiyolojik Kalitesi

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Özet: Kars'da farklı marketlerde satılan 35 adet paketlenmemiş, 35 adet vakum paketlenmiş toplam 70 adet Frankfurter tip sosis örneği ve 30 adet paketlenmiş salam örneği mikrobiyolojik kaliteleri bakımından analiz edildi. Yapılan analizlerde Aerob mezofil genel canlı, koliform, enterobakteri, enterokok, *Pseudomonas* spp., stafilokok ve mikrokok, *C. perfringens*, maya ve küf düzeyi saptandı. Ayrıca tüm örneklerde *Salmonella* spp.nin varlığı araştırıldı. Bütün salam ve vakum paketlenmiş Frankfurter tipi sosislerde Aerob mezofil genel canlı, koliform, enterobakteri, enterokok, *Pseudomonas* spp., stafilokok ve mikrokok, maya ve küf sayısı $< 2.0 \times 10^2$ kob/g olarak saptandı. Salam ve vakum paketlenmiş Frankfurter tipi sosislerin hiç birinden *Salmonella* spp. saptanamadı. *C. perfringens*, 3 salam örneğinde sınır seviyesi olan 10^2 kob/g düzeyinde saptanırken (TSE 979), 4 salam örneğinde 10^3 kob/g düzeyinde saptandı. Vakum paketlenmiş Frankfurter tip sosilerde ise *C. perfringens* 2 örnekte sınır seviyesi olan 10^2 kob/g düzeyinde saptanırken (TSE 980), 3 örnekte 10^3 kob/g düzeyinde saptandı. Paketlenmemiş Frankfurter sosislerde ise ortalama olarak Aerob mezofil genel canlı ve *Pseudomonas* spp. sırasıyla 1.3×10^4 ve 6.0×10^4 kob/g olarak saptandı. Enterokok, enterobakteriler, koliform, stafilokok ve mikrokok, maya ve küf düzeyleri ortalama değerleri sırasıyla, 1.1×10^3 , 2.8×10^3 , 2.4×10^3 , 2.6×10^3 ve 1.9×10^3 kob/g olarak saptandı. Paketlenmemiş 18 Frankfurter tipi sosis'den *E. coli* saptandı. 21 örneğin 13'ünden koagülaz (+) stafilokok izole edildi, bunların 11'i *S. aureus* olarak tanımlandı. *C. perfringens* düzeyinin çoğunlukla $< 2.0 \times 10^2$ kob/g olmak üzere $< 2.0 \times 10^2$ ile 1.0×10^4 kob/g arasında değiştiği saptandı. Paketlenmemiş 35 Frankfurter tipi sosis'den 6'sından *Salmonella* spp. izole ve tanımlandı.

Anahtar Kelimeler: Emülsifiye sucuk, frankfurter sucuk, mikrobiyolojik kalite, salam

Microbiological Quality of Emulsified Type Meat Products (Salami-Frankfurter Sausage) Marketed in Kars

Abstract: Seventy Frankfurter type sausage samples including 35 unpackaged and 35 vacuum packaged, and 30 unpackaged salami samples from different retail markets in Kars, were analyzed for their microbiological quality. Tests were performed including the counts of aerob mesophile bacteria, coliforms, *Enterobacteriaceae*, *Enterococci* spp., *Pseudomonas* spp., *Staphylococci* and *Micrococci* spp., *Clostridium perfringens* (*C. perfringens*), yeast and mould the presence of *Salmonella* spp. were also analyzed on all the samples examined. Aerob mesophile bacteria, coliforms, *Enterobacteriaceae*, *Enterococci* spp., *Pseudomonas* spp., *Staphylococci* and *Micrococci* spp., yeast and mould counts were detected at the counts of $< 2.0 \times 10^2$ cfu/g in all salami and vacuum packaged Frankfurter type sausages. *Salmonella* spp. were not detected on any of the salami and vacuum packaged Frankfurter type sausages. Three salami samples contained *C. perfringens* at the level of 10^2 cfu/g, which is in the limit level (Turkish Standards Institute, TSE 979) and four salami samples had *C. perfringens* at the count of 10^3 cfu/g. Of the vacuum packaged Frankfurter type sausage samples, two samples contained *C. perfringens* at the level of 10^2 cfu/g, which is in the limit level (TSE 980) and three samples had 10^3 cfu/g of *C. perfringens*. Of the unpackaged Frankfurter type sausages, the mean counts per g for total mesophile bacteria and *Pseudomonas* spp. were 1.3×10^4 and 6.0×10^4 cfu/g, respectively. The mean counts for *Enterococci* spp., *Enterobacteriaceae*, coliform, *Staphylococci* and *Micrococci* spp., yeast and mould counts were 1.1×10^3 , 2.8×10^3 , 2.4×10^3 , 2.6×10^3 and 1.9×10^3 cfu/g, respectively. Microbial load of the 18 unpackaged Frankfurter type sausage samples were *E. coli* positive. Out of 21 samples, 13 were coagulase (+) *Staphylococci* spp. and 11 of these were *S. aureus*. *C. perfringens* was at a load ranging from $< 2.0 \times 10^2$ to 1.0×10^4 cfu/g, with the most being less than $< 2.0 \times 10^2$ cfu/g. Six *Salmonella* spp. were isolated and identified out of 35 unpackaged Frankfurter type sausages.

Key words: Emulsified sausage, frankfurter sausage, microbiological quality, salami

Introduction

Eating habit in Turkey is changing with the life style as in many other developing countries. There has been an increase in the consumption of ready-to-eat foods such as Frankfurter-type sausages (as known 'sosis' in Turkey) and salami in Turkey. Salami and sausages (Frankfurter, Bologna, Vienna) are traditional European foods

made using ground meat combined with fat, herbs, spices and other curing ingredients, e.g. nitrite and salt. Frankfurter type sausage and salami mixtures are filled into casings and subjected to the various applications as follows: drying (appr. 20-25 min at 40-45 °C), smoking (20 min at 60-65 °C) and steaming (1-1.5 h at 70-80 °C for salami; 2-2.5h at 70-80 °C for Frankfurter type sausages). Following the steam application, products are kept in cold water at + 4°C (30). Although the microbial load of the final product is reduced by such processes as

smoking and heating treatment, ingredients such as spices and herbs used during the production directly effect the microbial quality of the end product. Previous epidemiological studies (1,17,18,27,28) on the spices in Turkey have raised concerns over the safety of spices sold in retail markets. Recent studies (8,10,11,20,23) have suggested that cooking time during the processes, storage conditions of the products and possible secondary contamination may cause a risk for human health. The objectives of this investigation, therefore, were initially to determine the microbiological quality of the vacuum packaged and unpackaged Frankfurter type sausage and salami samples sold in the markets and supermarkets of Kars, secondly to document the presence or absence of bacterial pathogens in Frankfurter type sausage and salami samples.

Materials and Methods

Sampling: A total of 70 Frankfurter type sausage samples (35 from unpackaged and 35 from vacuum packaged) and 30 non vacuum packaged salami samples were purchased from the supermarkets in Kars. The samples were collected into sterile polyethylene bags and transported to the laboratory in a cooling box and analysed within 4 hours.

Microbiological analysis: Microbiological analyses of the samples were carried out in accordance with the methods of Vanderzant and Splittstoesser (29) and Anon (5). Each sample in quantity of 25 g was aseptically weighted and blended in a stomacher for 2 min. with 225 ml of sterile buffered peptone water (BPW) (Oxoid CM509). Further decimal dilutions were prepared with the same diluent. Drope plaque method was used for the enumeration of aerob mesophile bacteria, coliforms, *Enterobacteriaceae*, *Enterococci* spp., *Pseudomonas* spp., *Staphylococci* and *Micrococci* spp., *C. perfringens*, yeast and mould counts. Aerob mesophile bacteria counts (30°C / 24-48 h) was performed using Plate Count Agar (Oxoid CM 325). *Pseudomonas* spp. were isolated on Pseudomonas Agar (Oxoid CM559+Suppl., Oxoid SR 0103) (30°C / 24-48 h). Colonies were then tested with oxidase test strips (Oxoid BR 63). Slanetz and Bartley Medium (Oxoid CM377) was used for the isolation of *Enterococci* spp. (37°C/24-48h). Purple-red colonies having precipitation zones on SBM were counted *Enterobacteriaceae* were isolated on Violet Red Bile Glucose Agar (Oxoid CM485) (37°C / 24-48 h). At the end of incubation, all purple-reddish colonies

were counted. Coliform count and *E. coli* were done using Violet Red Bile Lactose Agar (Oxoid CM107) at 37°C for 24-48 h. After incubation, colonies giving purple-reddish colour with precipitation zones were streaked onto Endo Agar (Oxoid CM479) and incubated at 37°C for 24-48 h. Colonies with characteristic greenish metallic colour were subjected to IMVIC test to identify *E.coli*. Colonies displaying (+, +, -, -) or (-, +, -, -) results were accepted as *E.coli*. *Staphylococci* and *Micrococci* spp. were isolated on Baird Parker Agar (Oxoid CM275+Egg yolk tellurite, Oxoid, SR54) (37°C / 24-48 h). Typical black colonies surrounded by a transparent zone and small-brown colonies without zone were selected for further characterisation tests. Presumptive *Staphylococci* and *Micrococci* spp. colonies were seeded into Brain Heart Infusion Broth (Merck 1.10493). After 24-48 h incubation at 37°C, the coagulase test (Merck 1.3306) were performed and coagulase (+) giving colonies were subjected to the DNase test (Merck 1.10449). Presumptive *S. aureus* colonies were also tested with Gram stain test, catalase test and lysostaphin test. For yeast and mould, Rose Bengal Chloramphenicol Agar (Oxoid CM549+Chloramphenicol Selective Suppl., Oxoid SR 78) was used (25°C/4-5 days). The presence of *Salmonella* spp. in samples were analysed using the method of surface streaking. For *Salmonella* spp., 25 g of each sample was aseptically weighted and blended in a stomacher for 2 min with 225 ml of sterile BPW (Buffered Peptone Water), and incubated (37°C/24 h). Subsequently, 0.1 ml of incubated BPW was transferred into a tube containing 9.0 ml of Rappaport Vassiliadis Broth (Merck 1.07700) (43°C / 24 h) and then streaked onto Salmonella Shigella Agar plates (SS) (Merck 1.07667) and incubated at 37°C for 24-48 h. The plates were examined after 24-48 h. Typical colonies were restreaked onto Triple Sugar Iron Agar (TSI) (Merck 103915) and Lysine Iron Agar (Merck 111640). Typical *Salmonella* spp. colonies having black centred with halo zones were seeded into TSI and LIA and incubated at 37°C for 24 h. Considering the utilisation of lactose, sucrose and glucose in TSI and decarboxylation activity in LIA, presumptive *Salmonella* spp. colonies were then subjected to the serological test (Oxoid FT 203). Tryptose Cycloserine Agar (TSC) (Merck 11972 + Flurocult TSC Agar Suppl. Merck 1.04032) was used for the isolation of sulphide reducing anaerobes (37°C / 24-48 h). Typical black colonies were tested with Gram stain, catalase test, reverse CAMP, acid phosphatase test, NO₃ test, motility and lactose gelatin test.

pH measuring: The pH of each sample varied between 6.1 to 6.6 for sausage samples and 6.0 to 6.6 for salami samples.

Results

In all salami and vacuum packaged Frankfurter-type sausage samples, the counts of total aerob mesophile, *Pseudomonas* spp., *Enterococci* spp., *Enterobacteriaceae*, coliform, *Staphylococci* and *Micrococci* spp., yeast and mould count were lower than 2.0×10^2 cfu/g, except *C. perfringens*. *Salmonella* spp. were not detected in any samples of salami and Frankfurter type sausage examined. The counts of *C. perfringens* in salami samples revealed at a level of 10^2 cfu/g in three samples and 10^3 cfu/g in four samples. The level of *C. perfringens* in vacuum packaged Frankfurter type sausage samples was similar to salami samples as shown in Table 1, 10^3 cfu/g in three samples and 10^2 cfu/g in two samples.

Of the unpackaged Frankfurter-type sausage samples, the mean count of for total mesophile bacteria and *Pseudomonas* spp. were 1.3×10^4 and 6.0×10^4 cfu/g, respectively. The mean counts for *Enterococci* spp, *Enterobacteriaceae*, coliform, *Staphylococci* and *Micrococci* spp., yeast and mould count were 1.1×10^3 , 2.8×10^3 , 2.4×10^3 , 2.6×10^3 and 1.9×10^3 cfu/g, respectively. Twenty-five (71.42 %) of the total 35 unpackaged Frankfurter type sausage samples analysed contained coliform bacteria in which eighteen of the 25 were found positive for *E. coli*. Of twenty-one (60.0%) presumptive *Staphylococcus* spp. from unpackaged Frankfurter type sausage samples, 13 were coagulase (+) *Staphylococci* spp. and 11 of these were identified as *S. aureus*. *C. perfringens* was isolated from 35 unpackaged Frankfurter type sausage samples. Two samples contained *C. perfringens* at the number of 10^2 cfu/g, while four samples contained 10^3 cfu/g of *C. perfringens*. The other three samples revealed this pathogen at a level of 10^4 cfu/g. Among the other 26 salami samples (74.29 %), *C. perfringens* was counted in the range numbers of $< 2.0 \times 10^2$ to 1.0×10^4 cfu/g, with the most being less than $< 2.0 \times 10^2$ cfu/g.

Table 1. Result of microbiological analysis of salami, Vacuum packaged and non-vacuum packaged Frankfurter sausage.

Table 1.

Sample	Level of microorganism cfu/g)	<i>Enterococci</i> spp.	<i>Enterobacteriaceae</i>	Coliform	<i>C. perfringens</i>	Aerob Mesophile counts	<i>Pseudomonas</i> spp.	<i>Staphylococci</i> and <i>Micrococci</i> spp.	Yeast and mould
Salami	<10 ²	30	30	30	23	30	30	30	30
	10 ²	-	-	-	3	-	-	-	-
	10 ³	-	-	-	4	-	-	-	-
Vacuum Packaged Frankfurter sausage	<10 ²	35	35	35	30	35	35	35	35
	10 ²	-	-	-	2	-	-	-	-
	10 ³	-	-	-	3	-	-	-	-
Non-vacuum packaged Frankfurter Sausage	<10 ²	19	6	10	26	-	6	14	9
	10 ²	5	3	2	2	6	10	2	4
	10 ³	8	18	16	4	19	16	11	17
	10 ⁴	3	8	7	3	6	1	8	5
	10 ⁵	-	-	-	-	4	2	-	-

Discussion

The initial microbial load of the meat directly effects the microbial quality of the final products (like salami and Frankfurter type sausage) (12,13,15,22,26). Time-dependent heating exposures during the production of salami and Frankfurter type sausages (sisis) largely reduce the vegetative forms of the microorganisms, nonetheless, spore bearing bacteria may pose risks in case of consuming of these kind of ready-to-eat types of foods (24,25). In the present study, pathogenic microorganisms were not detected in salami and vacuum packaged Frankfurter type sausage samples with the exception of *C. perfringens*. Absence of other pathogenic bacteria seems to be acceptable as this may be consequences of drying during the production processes, fumigation, steaming, advanced production technology, proper sanitation and disinfection rules, increasing the shelf life of the product and applied vacuum process for the best quality of the products.

Bacterial cells exposed to different physical and chemical treatments suffer injury that could be reversible in food materials during storage. Injury has been observed for many bacterial cells can repair in a medium containing the necessary nutrients under conditions of optimum pH and temperature leading to outbreaks of foodborne disease and food spoilage (9). *C. perfringens* is ubiquitous organism which is found in human and animal excreta and in soil and dust, can readily contaminate food. Cooked meat provide appropriate anaerobic environment, and spores of the organisms can survive cooking heat. Unrefrigerated storage may provide optimum temperature for germination of the spores during the slow period of cooling and proliferation may occur with the subsequent production of toxin. Toxin is only formed by actively sporulating organisms, and thus the temperature conditions allowing the spores to develop into vegetative forms are critical. Cooling in a refrigerator will reduce temperatures sufficiently and quickly so that the foods will pass enough throughly the critical range to prevent germination taking place (7). Although the addition of nitrite-salt into meat mixture has a protective mechanism against the botulinismus during the production, the presence of *C. perfringens* in salami and vacuum packaged Frankfurter-type sausages may be the consequences of the microbial quality of the either raw meat material and/or the initial load of the spices and herbs (14).

Spices are important vectors for various microorganisms implicating possible health problems for consumers as well as quantity and shelf-life problems for food studies on the microbial quality of spices (1,7,17,18,27,28). Our results indicate a much higher incidence in emulsified type sausage and salami samples than those reported by Gokce et al. (19), who found that the incidence of *C. perfringens* in 41 salami and 45 sausage (sisis) samples amounted to 7.3 % and 13.3 %. Consequently, with such high incidences of contamination as we detected in our study, may pose a food poisoning risk for the people consuming of these types of products.

Considering all microbiological parameters including Aerob mesophile bacteria, *Pseudomonas* spp., *Enterococci* spp., *Enterobacteriaceae*, coliform, *Staphylococci* and *Micrococci* spp., yeast and mould, *E. coli*, *S. aureus*, *Salmonella* spp. and *C. perfringens* level in unpackaged Frankfurter type sausage samples are notably higher than in salami and vacuum packaged Frankfurter type sausages. Upon observing the points of the sales of the vacuum packaged and non vacuum packaged, poultry and red meat products were kept in the same place and also the workers handled up the both types of products using the same gloves. The contamination of the manufactured product may be attributed to the inappropriate storage conditions or lack of personal hygiene (16). The microbial quality of the vacuum packaged sausages is significantly lower than that of non vacuum packaged Frankfurter type sausage and salami samples. This is much possibly attributed to the protective mechanism of vacuum packaging material (21).

In Turkey, there have been several reports indicating that emulsified type meat products have been shown to have large numbers of microorganisms present, including pathogens. Agaoglu (2) analyzed the equal amount of 20 vacuum packaged salami and Frankfurter sausage samples. Apaydin et al. (6) examined the microbiological quality of the 30 salami samples. In those studies, samples were subjected to analysis of: Aerob mesophile counts, yeast and mould, *Enterobacteriaceae*, coliforms, *E. coli*, *Staphylococci* and *Micrococci* spp., *S. aureus*, *Pseudomonas* spp. and *Enterococci* spp. In these two separate studies, the counts of *Enterobacteriaceae*, coliform, *Enterococci* spp., *Staphylococci* and *Micrococci* spp., yeast and mould show a similarity with our study.

The present data revealed that microbiological quality of twenty-six salami samples (86.6 %) and

thirty-two vacuum packaged Frankfurter type sausages (91.42 %) sold in the market and supermarkets of the Kars seems to be satisfactory based on Turkish Standards Institute (TSE) standard for salami (TS 979) (3) and sosis (TS 980) (4). Although Aerob mesophile counts in unpackaged sausages is satisfactory according to sausage (TSE 980) standard, other microbiological parameters are not completely in accordance with TS 980 standard.

Therefore, particular attention should be given regarding the Hazard Analysis Critical Control Point (HACCP) system, during the production, and storage of unpackaged sausage, particularly considering *C. perfringens* a spore forming bacteria. Keeping unpackaged (non vacuum) products in a separate place from raw meat and raw meat products are advisable in order to prevent cross contamination and infection or toxication due to pathogenic microorganisms. Additionally, workers in food production should be trained and Good Manufacturing Practise (GMP) should be followed for maintaining good hygiene, in terms of public health.

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