

Presence of *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157 and Nitrate-Nitrite Residue Levels in Turkish Traditional Fermented Meat Products (Sucuk and Pastırma) ^[1] ^[2]

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Abstract

Turkish sucuk and pastırma are traditional meat products commonly consumed in Turkey. These products are generally known as dry fermented meat products (FMP), fermented and ripened naturally. Curing is a preparation method for FMPs used for prolonging shelf life. As well as additives such as nitrate and nitrite are used to obtain the desired colour and flavour, also inhibit the mentioned bacteria. Despite the advantages of the curing agents, FMPs may pose a risk for human health via uncontrolled (out of limits) usage. The present study was conducted to investigate the incidence of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157 and nitrate-nitrite contents in 132 sucuk and 66 pastırma samples collected from producers and retailers in Istanbul, Adapazari, Afyon and Kayseri. *Salmonella* spp. and *L. monocytogenes* were detected 2.52% and 2.02% in all samples, respectively. All samples were negative for *E. coli* O157. The nitrate level of sucuk and pastırma samples were found was in the acceptable range. Only, 5 of sucuk samples exceeded the nitrite limit value. The results indicate that meat products may be contaminated with pathogens and nitrosamines can be present in meat products. Furthermore, the essential precautions should be taken to apply sanitation procedure and improve the quality of production technology.

Keywords: Sucuk, Pastırma, *Listeria monocytogenes*, *Salmonella* spp., Nitrate-nitrite

Geleneksel Türk Fermente Et Ürünlerinde (Sucuk ve Pastırma) *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157 ve Nitrat-Nitrit Varlığı

Özet

Türk tipi sucuk ve pastırma Türkiye'de yaygın olarak tüketilen geleneksel et ürünleridir. Doğal olarak fermente olup olgunlaşan bu ürünler genellikle fermente edilerek kurutulmuş et ürünleri olarak bilinirler. Kürlenme fermente edilerek kurutulmuş et ürünlerinde kullanılan bir hazırlama metodu olup raf ömrünü uzatmak amacıyla kullanılır. Nitrat ve nitrit gibi katkı maddeleri de arzu edilen renk ve aromanın şekillenmesini sağlarken aynı zamanda bazı bakterilerin üremesinin inhibe edilmesi için de kullanılır. Kürlenme ajanlarının avantajlarına rağmen, fermente edilerek kurutulmuş et ürünlerinde kontrolsüzce (limit değerlerin üzerinde) kullanımları sağlık risklerine sebep olabilir. Bu çalışma İstanbul, Adapazari, Afyon ve Kayseri'deki perakende satış noktaları ve üreticilerden toplanan 132 sucuk ve 66 pastırma örneğinde *Salmonella* spp., *Listeria monocytogenes* ve *Escherichia coli* O157 varlığını ve nitrat-nitrit içeriğini tespit etmek için yürütüldü. *Salmonella* spp. ve *L. monocytogenes* sırasıyla %2.52 ve %2.02 olarak tespit edildi. Hiçbir örnekte *E. coli* O157 tespit edilemedi. Sucuk ve pastırma örneklerinin nitrat düzeyi kabul edilebilir düzeyde bulundu. Sadece sucuk örneklerinin beşinde nitrit limit düzeyinin aşıldığı tespit edildi. Sonuçlar et ürünlerinin patojenlerle kontamine olabileceğini ve et ürünlerinde nitrozaminlerin bulunabileceğini göstermiştir. Buna ek olarak, uygulanan sanitasyon prosedürlerinde zorunlu tedbirler alınmalı ve üretim teknolojisinde kalite iyileştirilmelidir.

Anahtar sözcükler: Sucuk, Pastırma, *Listeria monocytogenes*, *Salmonella* spp., Nitrat-nitrit



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INTRODUCTION

Turkish type dry fermented sausage (sucuk) and pastirma are traditional meat products widely consumed in Turkey. These products are known as dry fermented meat products (FMP) manufactured by natural fermentation and generally consumed without cooking^[1-3]. Despite the fermentation periods the foodborne pathogens that can be present in the gastrointestinal tract of food-producing animals are potential sources of risk for human health^[4]. These organisms are subsequently transferred to meat products and people due to poor hygiene, sanitation conditions and handling procedures during slaughtering and production^[5].

Curing is a traditional culinary technique is used for prolonging the shelf life of meat products^[6]. The curing agents nitrite and nitrate not only help producers obtain the desired flavour and colour; but also have inhibitor effect on several pathogen microorganisms in fermented meat products^[7]. Despite their technological and safety advantages, high intake of nitrate-nitrite constitutes a risk to human health, in rare occasions causing allergenic effects and carcinogenic nitrosamines^[8,9].

The present study was undertaken to determine the prevalence of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157 and the contents of nitrate-nitrite in sucuk and pastirma obtained from retail markets and producers in Istanbul, Adapazari, Afyon and Kayseri, the major sucuk and pastirma producing cities in Turkey.

MATERIAL and METHODS

Sample Collection: Sucuk and pastirma samples were collected at intervals between March 2012 and February 2013. A total of 132 sucuk and 66 pastirma samples were examined for the presence of *Salmonella* spp., *L. monocytogenes*, *E. coli* O157 and nitrate-nitrite contents. Samples were obtained from producers and retailers in Istanbul, Adapazari, Afyon and Kayseri. All samples were collected in their original packages and transferred to the laboratory at 4°C.

Microbiological Analysis: For isolation of *Salmonella* spp., pre-enrichment was done by suspending 25 g of sample in 225 ml buffered peptone water (BPW - Oxoid CM0509), followed by incubation at 37°C for 16 to 20 h. A 0.1 ml sample of the mixture was transferred to Rappaport-Vassiliadis (RV - Oxoid CM0866) and Muller Kaufmann Tetrathionate Broth (MKTTn - Oxoid CM0343) and they were incubated for 24 h at 42°C. Samples were streaked on Hectoen Enteric Agar (Oxoid CM0419) and XLD Agar (Oxoid CM0469) after incubation and incubated an additional 24 h at 35°C. The typical colonies were identified by biochemical tests and confirmed with *Salmonella* antiserum (O and H-Vi polyvalent antiserum)^[10].

For detection of *L. monocytogenes*, 25 g food samples were mixed with 225 ml of Listeria Enrichment Broth (Oxoid CM0862) containing Listeria Selective Supplement (Oxoid SR 141). Samples were homogenized in a stomacher bag for 60 sec and incubated at 32°C for 24 h. 0.1 ml portion of the enrichment broth was streaked onto Chromogenic Listeria Agar (Oxoid CM1080) supplemented with Listeria Selective Supplement (Oxoid SR0227) and Listeria Differential Supplement (Oxoid SR0228). After incubation, typical colonies were transferred to Tryptic Soy-Yeast Extract Agar (Oxoid CM0131) and incubated for 24 to 48 h at 30°C. These colonies were verified by Gram's staining, catalyses reaction, tumbling motility at 25°C, Methyl Red-Vogues Proskauer (MR-VP) reactions, CAMP test, nitrate reduction and fermentation of sugars^[11].

For detection of *E. coli* O157, each sample was examined by combining 25 g with 225 ml of modified Tryptone Soya Broth (Oxoid CM0989) into a stomacher bag, homogenized for at least 2 min and incubated at 37°C for 24 h. Enriched cultures were streaked onto Sorbitol MacConkey Agar (Oxoid CM0813) supplemented with Cefixime Tellurite Selective Supplement (Oxoid SR172) and incubated at 37°C for 18 to 24 h. Following the incubation period, the colourless colonies were tested by *E. coli* O157 latex kit (Oxoid DR0620)^[12].

Determination of Nitrate and Nitrite Content: Nitrate and nitrite concentrations in the samples were determined by the HPLC method based on the Nordic Committee on Food Analysis Method No. 165. The solution was injected onto the Shimadzu LC10 chromatograph. Nitrate and nitrite were separated by an Alltech C18 column and measured with an ultraviolet light detector at a wavelength of 205 nm. The limit of quantification for both ions was 5mg/kg⁻¹; the measurement uncertainty (U) at a concentration of 100 mg/kg was 12 mg/kg (k=2, normal)^[13].

RESULTS

Salmonella spp. and *L. monocytogenes* were detected at 1.52% and 1.52% in sucuk and 4.55% and 3.03% in pastirma samples, respectively (Table 1). All samples were negative for *E. coli* O157. According to Turkish Food Codex^[14], the presence of *Salmonella* spp. and *E. coli* O157 in 25 g of raw beef or ground beef as well as the presence of *Salmonella* spp. and *L. monocytogenes* in 25 g of sucuk is unacceptable. The results of nitrate and nitrite concentrations are shown in Table 2.

DISCUSSION

The presence of *Salmonella* spp. in fermented meat products have been examined in several studies. Oksuztepe et al.^[15] demonstrated that *Salmonella* spp. was isolated from 3.0% of products. Erdogru and Ergun^[16] reported that 1.66% was found to be the positive for *Salmonella*

Table 1. Prevalence of *Salmonella* spp., *L. monocytogenes* and *E. coli* O157 in sucuk and pastırma samples**Tablo 1.** Sucuk ve pastırma örneklerinde *Salmonella* spp., *L. monocytogenes* ve *E. coli* O157 prevalansı

Products	<i>Salmonella</i> spp.		<i>L. monocytogenes</i>		<i>E. coli</i> O157	
	+	%	+	%	+	%
Sucuk (n=132)	2	1.52%	2	1.52%	-	-
Pastırma (n=66)	3	4.55%	2	3.03%	-	-
Total (n=198)	5	2.53%	4	2.02%	-	-

+: number of positive samples

at very low levels together with very high levels of competitor organisms which is making it difficult to detect.

Regarding the contamination rate of sausages, the results in this study were low. The reason for the low contamination rate is likely due to the fermentation process which reduces the number of pathogens during curing or storing time. Lactobacilli play an important role in the protection against the pathogens and in the development of flavour by producing lactic acid [30]. The presence of lactic acid accelerates pH decline and water activity, improving the safety and stability of these products [31].

Table 2. The results of nitrate and nitrite concentrations in sucuk and pastırma samples**Tablo 2.** Sucuk ve pastırma örneklerinde nitrat ve nitrit konsantrasyon sonuçları

Parameters	Sucuk (n=132)			Pastırma (n=66)			Satisfactory Limit by TFC	Unacceptable Samples (Sucuk/Pastırma)
	Min	Max	Mean	Min	Max	Mean		
Nitrate (mg/kg)	28.10	174.62	87.28	64.12	187.66	108.83	250	(0/0)
Nitrite (mg/kg)	6.41	90.02	24.83	4.26	46.28	17.33	50	(5/0) (3.79%)

TFC: Turkish Food Codex

spp. The findings were highly consistent with these results (1.52% in sucuk and 4.55% in pastırma). In other studies, no *Salmonella* spp. was isolated [17,18]. Contrarily, two studies which reported higher results (5.0% and 7.0%) than this study were those of Kok et al. [19] and Siriken et al. [20]. They explained the high *Salmonella* spp. prevalence with varying hygiene applications at slaughterhouses and meat markets. Different detection rates may originate from detection methods, sampling procedures and the sanitation applications.

The prevalence of *L. monocytogenes* in the fermented samples tested in this study was lower than those detected by Farber et al. [21] in Canada (20.0%), Cantoni et al. [22] in Italy (13.0%), Jemmi et al. [23] in Switzerland (15.0%) and Colak et al. [24] in Turkey (11.6%). On the other hand, Mena et al. [25] reported the prevalence rate of *L. monocytogenes* was 3.7% in 27 Spanish fermented sausages while Ciftcioglu and Ugur [26] detected only 2.0% in their Turkish samples. The findings of this study showed similarity with the mentioned results. Differences between the findings from these studies can be related to production techniques, contaminations after production process, preservation conditions and inadequate personal hygiene. In this study *Listeria* species were also detected in 9 samples as *L. innocua* (in 5 samples), *L. seeligeri* (in 2 samples) and 2 *L. welshimeri* (in 2 samples).

According to the results from this study, *E. coli* O157 was not detected. Similar results were reported by Ferreira et al. [27] in Portugal and Siriken et al. [20] in Turkey. In Argentina, 4.8% of 83 fresh sausages and 3.3% of 30 dry sausages were contaminated with *E. coli* O157 [28]. Tilden et al. [29] reported that *E. coli* O157:H7 was linked to consumption of fermented salami in USA. *E. coli* O157 presents sporadically

In the present study, the nitrate concentrations of samples were found a mean of 87.28 mg/kg in sucuk and 108.83 mg/kg in pastırma. The mean value of nitrite concentrations was 24.83 and 17.33 with a range of 6.41 to 90.02 mg/kg and 4.26 to 46.48 mg/kg in sucuk and pastırma samples, respectively. Today, many countries enforce production limits of nitrate and nitrite. In general, 250 mg/kg of nitrate and 50 mg/kg of nitrite are to be permitted for all meat products [32].

In the present study, the highest rates of *Salmonella* spp. (4/5) and *L. monocytogenes* (3/4) were detected in June, July and August which have the highest temperatures in Turkey. Temperature is considered the most critical factor for the microbial quality of meat at the stage of manufacture, distribution and consumption. Microbial growth is seen corresponds directly with temperature increase [33].

Nitrate is necessary in a long curing process to act as a source of nitrite [34]. In the present study, the nitrate level of sucuk and pastırma samples were found all to be in the acceptable range. The findings corroborated studies of Sanlı and Kaya [35] and Servi [36]. On the contrary, higher values were found by Sezer et al. [37] and Soyutemiz and Ozenir [38]. Five (3.79%) of the sucuk samples exceeded the nitrite limit value, but the nitrite level in pastırma was within the satisfactory limit. Similar results were reported by Aksu ve Kaya [39], El-Khateib et al. [40] and Sancak et al. [41].

In conclusion, the result of this study confirmed that meat products can become contaminated pathogens such as *Salmonella* spp. and *L. monocytogenes*, which can cause serious public health problems. The quality of raw meat, heat treatment of the meat, the activity of the starter culture, the salting process and the storage conditions are

the most important points of control for the prevention of growth/survival of undesirable microorganisms during the manufacturing process [42]. Therefore, it is essential and beneficial to apply good hygienic practice, good manufacturing practice, HACCP and to develop standard production procedures for controlling foodborne pathogens and enhancing the safety of food. Further studies to identify the risk factors for the presence of pathogens in the production of sucuk and pastırma should be carried out.

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