International Food Research Journal 27(1): 39 - 46 (February 2020)

Journal homepage: *http://www.ifrj.upm.edu.my*

Determination of some foodborne pathogens and residual nitrate and nitrite in traditional fermented sausages in Turkey

*Gürbüz, S. and Çelikel Güngör, A.

Department of Gastronomy and Culinary Arts, Faculty of Tourism, Mardin Artuklu University, Mardin, Turkey

Article history

Received: 3 July 2019
Received in revised form: 30 November 2019
Accepted: 2 December 2019

Keywords

fermented sausage, foodborne pathogens, residual nitrate, residual nitrite, Turkey

Abstract

The present work was performed to examine the traditionally fermented sausages in terms of food safety by investigating the presence of *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157, and *Staphylococcus aureus*, as well as the residual nitrate and nitrite content. For this purpose, a total of 45 fermented sausage samples, 32 of which were produced and sold in butcher shops and 13 by different industrial companies, were analysed. *Listeria monocytogenes*, *Salmonella* spp., and *E. coli* O157 were not detected in any of the samples, whereas *S. aureus* was detected in 5 (11.1%) samples. The amount of residual nitrate was found between 4.30 and 62.59 mg/kg in 39 (86.66%) of the analysed samples, and the amount of residual nitrite was found between 8.94 and 55.95 mg/kg in 8 (17.77%) of the samples. Detection of *S. aureus*, which is an important pathogen for food poisoning, and presence of residual nitrate and nitrite in fermented sausages should be taken into consideration due to possible public health risks that it may cause. Therefore, raising the awareness of the manufacturers about the control of production processes and the effectiveness and continuity of the controls by the competent authorities are of significant importance.

Introduction

Fermented sausage is a traditional meat product that is produced mainly from beef at homes, butcher shops, and by industrial companies; and it is widely consumed in all regions of Turkey. Nitrite, salt, and starter culture that are used in the preparation of fermented sausage, fermentation, and ripening/drying stages can inhibit the occurrence or can decrease the number of several spoilage and foodborne pathogen bacteria (Junttila *et al.,* 1989; Campelos *et al.,* 2016; EFSA and ECDC, 2017). However, microbial food safety is not always guaranteed due to differences in the variety and quantity of additives and/or ingredients used, as well as differences in processing steps such as fermentation and storage (Jemmi *et al.,* 2002; Swaminathan *et al.,* 2007).

The presence of *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157, and *Staphylococcus aureus* in fermented sausages has been reported in many studies (Chinen *et al.,* 2001; Jemmi *et al.,* 2002; Kök *et al.,* 2007; Büyükünal *et al.,* 2016). Although listeriosis caused by *L. monocytogenes* is a rare disease, *L. monocytogenes* is an important bacterium because it also causes meningitis, septicaemia, and meningoencephalitis, resulting in death in 20-30% of the cases (Jemmi *et al.,* 2002; Swaminathan *et al.,* 2007). In European Union (EU) countries, there were 2,536 confirmed human listeriosis cases in 2016 (EFSA and ECDC, 2017). *L. monocytogenes* is widely available in the environment, raw material of fermented sausage, and equipment (Jemmi *et al.,* 2002; Martin *et al.,* 2011).

Salmonella spp. contaminate meat, meat products, and other foods; and remain a leading cause of foodborne bacterial infections. It is widely present in the environment and often colonises the gastrointestinal tract of many animal species (D'Aoust and Maurer, 2007; Martin *et al.,* 2011). It is reported that salmonellosis is the second most common zoonoses among people in the EU (EFSA and ECDC, 2017).

Escherichia coli O157 is a foodborne pathogen that can cause haemolytic uremic syndrome characterised by haemolytic anaemia, thrombocytopenia, and kidney damage in humans (Rangel *et al.,* 2005). Conditions such as the use of contaminated meat in the production, short curing period, and the slow start of fermentation are favourable for the proliferation and maintenance of this bacterium in fermented sausage (Sartz *et al.,* 2008).

Staphylococcal food poisoning caused by the

*Corresponding author.
Email: semragurbuz@gmail.com

consumption of food contaminated with enterotoxins of coagulase positive *S. aureus* is one of the most common causes of gastroenteritis worldwide (Junttila *et al.,* 1989). Humans and animals infected by *S. aureus* and contaminated equipment used in food processing are the main sources of transmission (Hennekinne *et al.,* 2012).

Sodium and potassium salts of nitrate and nitrite are used as food additives. The legal maximum amount of nitrate and nitrite that may be added to fermented sausages during production is 150 mg/kg (EU, 2006). The use of nitrates is deemed necessary in traditional fermented sausages, which have a long ripening process (EFSA, 2003). The added nitrate is gradually reduced to nitrite by the effect of microorganisms (Honikel, 2008).

In fermented sausages, nitrite contributes in fixing of the characteristic colour, typical and distinctive flavour, and inhibits microbial growth. Although there is no direct relationship between residual nitrite content and the amount of nitrite added to the product, factors such as pH of the product, storage temperature, and the presence of ascorbate affect the residual nitrite content (EFSA, 2003). A significant amount of nitrite added to the product is reduced by chemical reactions during production and the remaining 10 - 20% residual nitrite decreases during storage and can be reduced to undetectable levels (Sindelar and Milkowski, 2012). Nitrite, a highly reactive substance, can be partially oxidised to nitrate, thus a significant amount of nitrate can be detected in meat products due to nitrite addition (Honikel, 2008).

The most common health effect of high consumption of nitrate is methemoglobinemia, which causes a decrease in the oxygen-carrying capacity of red blood cells due to the methaemoglobin formed by the interaction of haemoglobin in the blood with nitrite, which is the reduction product of nitrate (WHO, 2011). N-nitrosamine, which is formed as a result of the reaction of nitrite with secondary amines during storage of foods, has negative effects on health. Although evidence for the carcinogenic effect of nitrite in humans is limited, an association with an increased incidence of gastric cancer is reported and in the presence of conditions that cause endogenous nitrosation ingested nitrate or nitrite is classified as probably carcinogenic to humans (Group 2A) (IARC, 2010). Although dietary exposure to N-nitroso compounds is low, it is recommended to continue the efforts to reduce exposure by considering the genotoxic and carcinogenic potential of some of these substances (EFSA, 2010).

Fermented sausage is usually produced according to local traditions in small butcher shops in Turkey during the transition period from autumn to winter, without using a standard method, and is offered for sale in the shop where it is produced. The present work was carried out in order to evaluate the possible public health risks by investigating the presence of *L. monocytogenes*, *Salmonella* spp., *E. coli* O157, and *S. aureus*; and also the presence and levels of residual nitrate and nitrite in traditional fermented sausages, produced by industrial companies and butcher shops in Turkey.

Materials and methods

Sample collection

A total of 45 fermented sausage samples, which were produced from beef in 32 butcher shops and 13 industrial companies by traditional methods without thermal process, were purchased in Turkey between February and March 2018. Each sample was taken as sold to the customer with the original packaging, brought to the laboratory in cold box, and tested for the presence of *L. monocytogenes*, *Salmonella* spp., *E. coli* O157, and *S. aureus* as well as the presence and levels of residual nitrate and nitrite.

Isolation and identification of foodborne pathogens
L. monocytogenes

Samples were tested for *L. monocytogenes* following the method specified by ISO 11290-1:2017 (ISO, 2017a).

Salmonella spp.

Samples were tested for *Salmonella* spp. following the method specified by ISO 6579-1:2017 (ISO, 2017b). The suspected colonies were tested with VITEK 2 for confirmation and identification.

E. coli O157

Samples were tested for *E. coli* following the method specified by TS EN ISO 16654 (TSI, 2003).

S. aureus

Samples were tested for *S. aureus* following the method specified by TS 6582-1 EN ISO 6888-1 (TSI, 2001). Colonies with black appearance and surrounded by zones was considered coagulase-positive *S. aureus* suspicious colonies. Typical colonies were verified with dry SPOT STAPHYTECT PLUS (Oxoid, DR0100M) (Bridson, 2006).

Residual nitrate-nitrite analysis

The analysis of residual nitrate and nitrite content of the samples were performed by HPLC as specified in Nordic Committee on Food Analysis No: 165 (NMKL, 2000). HPLC conditions were set as follows and measurement uncertainty and results were evaluated accordingly: flow rate: 0.7 mL/min, UV detector wavelength: 205 nm, injection volume: 100 µL, HPLC Column: Rp-C18 column (7 µm, 4.6 mm × 150 mm), column temperature: 25°C. The limit of detection was 2.70 and 3.65 mg/kg for nitrate and nitrite, and the limit of quantification was 9.00 and 12.17 mg/kg, respectively. The measurement uncertainty was determined as 12% for nitrite and 18% for nitrate (k = 2, normal).

Statistical analysis

Statistical analysis of the data was performed by SPSS ver. 21 program. The difference between the presence frequency of nitrite and nitrate in fermented sausages produced in local butcher shops and industrial companies was analysed by using two sample *t*-test. $p < 0.05$ was considered as statistically significant.

Results

L. monocytogenes, *Salmonella* spp. and *E. coli* O157 were not detected in any of the samples, while *S. aureus* was detected in 5 (11.1%) of 45 samples. All the samples, in which coagulase-positive *S. aureus* was detected, were the fermented sausages produced in the butcher shops (Table 1).

The amount of residual nitrate was found between 4.30 and 62.59 mg/kg in 39 (86.66%) of 45 fermented sausage samples, and 8.94 and 55.95 mg/kg of residual nitrite was detected in 8 (17.77%) samples (Table 2, Figure 1).

The differences of presence frequency of both residual nitrate and nitrite between the fermented sausages produced in industrial companies and local butcher shops were found to be statistically significant (Table 2).

Table 1. Pathogenic microorganisms in fermented sausage samples.

Sample type	Sample number	*n* positive (%)			
		L. monocytogenes	*Salmonella* ssp.	*E. coli* O157	*S. aureus*
Local	32	0	0	0	5 (15.6)
Industrial	13	0	0	0	
Total	45	0	0	0	5 (11.1)

Table 2. The amount of residual nitrite and nitrate in fermented sausage samples (mg/kg).

Sample type	Sample number	Residual nitrate		Residual nitrite	
		n positive (%)	Mean ± SD (min - max)	*n* positive (%)	Mean ± SD (min - max)
All samples	45	39 (86.66)	25.09 ± 14.95 (4.30 - 62.59)	8 (17.77)	29.97 ± 15.28 (8.94 - 55.95)
Local	32	28 (87.5)[a]	18.61 ± 10.62 (4.30 - 39.04)	2 (6.25)[b]	10.48 ± 2.18 (8.94 - 12.03)
Industrial	13	11 (84.61)[a]	41.60 ± 11.29 (20.41 - 62.59)	6 (46.15)[b]	36.46 ± 11.12 (22.31 - 55.95)

p values: [a] = 0.004; [b] = 0.014.

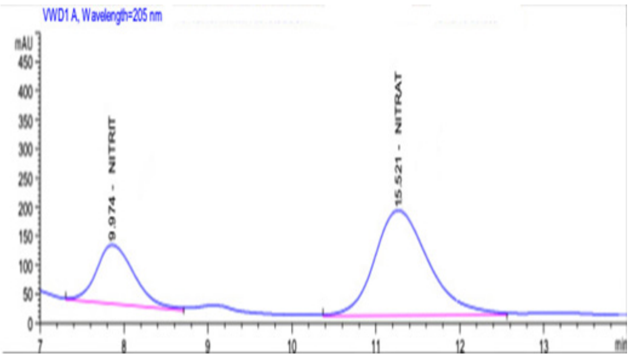

Figure 1. Chromatogram of residual nitrite and nitrate.

Discussion

Despite the effects of the additives used in fermented sausage, as well as the fermentation, ripening and drying steps, on reducing or inhibiting growth, pathogenic bacteria can be found in fermented sausages. Detection of pathogenic bacteria is one of the areas of interest of researchers because of public health risk.

It was reported in studies conducted in different parts of Turkey that *L. monocytogenes* were detected between 1.52 - 11.6% in fermented sausage samples (Öksüztepe *et al.,* 2011; Yalçın and Can, 2013; Büyükünal *et al.,* 2016). In contrast to these previous studies, as in the study of Yörük and Güner (2017), *L. monocytogenes* was not detected in any of the investigated samples. In a study of Johnson *et al.* (1988), it was found that *L. monocytogenes* did not proliferate during the fermentation and drying processes in typical hard salami production; however, it could survive if the bacterium was initially present at 10^3 CFU/g. It has been stated that salt, sodium nitrite, and pH, when used together, inhibit the growth of *L. monocytogenes* in the fermented sausages depending on the dosage (Junttila *et al.,* 1989). Jemmi *et al.* (2002) reported that they found *L. monocytogenes* in 15% of the fermented sausage samples imported and exported in Switzerland between 1992 - 2000 and that the risk of having this bacterium was related to the product category and enterprises. They could not determine any effect of origin of the country and season. In a study conducted in a small-scale enterprise in Spain by Martin *et al.* (2011), *L. monocytogenes* was found in the samples taken from different points during the production process, in the equipment used, in the raw materials, and in the final products. It was reported that *L. monocytogenes* was detected in 14.3% of traditionally dried and smoked fermented sausages by Campelos *et al.* (2016) in Portugal. It is noted that the growth of *L. monocytogenes* in suitably fermented sausages and other meat products can be prevented by the combined effect of low pH and a_w values and competitive flora (Johnson *et al.,* 1990; Jemmi *et al.,* 2002; Swaminathan *et al.,* 2007; EFSA and ECDC, 2017). The difference between the results of the present study and the previous ones may be caused by the types and amounts of the ingredients and additives, hygienic conditions in the production plant and differences due to the fermentation and storage conditions.

In the samples analysed in the present study, *Salmonella* spp. was not detected which was consistent with the results of the studies performed by Campelos *et al.* (2016) and Yörük and Güner (2017) (Table 1) and in contrast to the studies of Öksüztepe *et al.* (2011) and Büyükünal *et al.* (2016) who reported *Salmonella* spp. in 3 and 1.52% of the fermented sausage samples, respectively. In a study carried out by Martin *et al.* (2011), *Salmonella* spp. was not detected in the final product and on equipment, whereas it was detected in 23.7% of the raw material samples. It was reported by Hospital *et al.* (2014) that nitrite is effective in controlling *Salmonella* in fermented sausage, the pH of which is 5.2, after the fermentation and if there is not any nitrate/nitrite, *Salmonella* continues to grow. It was also reported in a study by Porto-Fett *et al.* (2008) that fermentation and drying processes in the production of fermented sausage causes a decrease in *S. typhimurium* by $\log_{10}$ 1.52 and 3.51 CFU/g, respectively in pH 5.3 and 4.8 levels. Unlike our study, the presence of *Salmonella* spp. in fermented sausage samples in some studies may be due to inadequate hygienic raw material and/or conditions during production and the test method used (Joseph *et al.,* 2001; Martin *et al.,* 2011).

Escherichia coli O157 was not detected in any of the samples examined (Table 1). In some other studies, the researchers have obtained similar results (Ferreira *et al.,* 2007; Campelos *et al.,* 2016). In a study conducted in Argentina by Chinen *et al.* (2001), it has been reported that *E. coli* O157:H7 was detected in 3.3% of 30 dried fermented sausage samples. Several factors, such as salt, nitrite, starter culture, water activity, and pH, may influence the maintenance of *E. coli* in fermented sausages (Holck *et al.,* 2011). It was reported in a study, which was conducted to develop models to predict the growth of pathogens under different conditions, that nitrite significantly inhibited the reproduction of *E. coli* (Milkowski *et al.,* 2010). It was also reported in another study conducted by Casey and Condon (2000) that inhibition rate of *E. coli* was faster in the presence of nitrite. Detection of *E. coli* 0157 in fermented sausages may be due to contamination of

Table 3. Compilation of data concerning residual nitrate and nitrite in fermented sausages in Turkey.

Reference	Total *n*	Province	Residual nitrate mg/kg	Residual nitrite mg/kg
Sancak *et al.* (2008)	40	Van	1.56 - 553.18	0.80 - 82.13
Sezer *et al.* (2013)	30	Kars	14.88 - 943.71	0.46 - 196.90
Büyükünal *et al.* (2016)	132	Istanbul, Adapazari, Afyon, and Kayseri	28.10 - 174.62	6.41 - 90.02
Benli (2017)	36	Adana	34.86 - 161.08	58.65 - 216.63
Present work	45	Mardin	4.30 - 62.59	8.94 - 55.95

the meat used in the production of fermented sausage, production under unhygienic conditions, and unsuitable production process (Sartz *et al.,* 2008).

In the present study, coagulase-positive *S. aureus* was found in 11.1% of the samples. It was found that the mean of coagulase-positive *S. aureus* was 4.7×10^4 CFU/g. It was reported for staphylococcal food poisoning incidents between 1969 - 1990 in United Kingdom that the level of *S. aureus* in food varied between none and 1.5×10^{10} CFU/g (median of 3.0×10^7 CFU/g) (Wieneke *et al.,* 1993). It was reported that the number of enterotoxigenic *S. aureus* higher than 10^5 and 10^6 CFU/g in food poses risk (EC, 2003). However, the number of coagulase-positive *S. aureus* found in the present study was lower than these values except for one sample. All the samples with coagulase-positive *S. aureus* were produced in butcher shops. This result is close to 12 and 10% of contamination ratios determined by Kök *et al.* (2007) and Öksüztepe *et al.* (2011), respectively. Our result is lower than the results of Yalçın and Can (2013) who found 16.6% contamination rate. Campelos *et al.* (2016) reported no *S. aureus* in any of the samples. The difference in the findings obtained by the researchers may be due to the use of raw material contaminated with *S. aureus* in the production of fermented sausage and the failure to perform the production under hygienic conditions and non-compliance of the staff with hygienic rules (Hennekinne *et al.,* 2012).

The amount of residual nitrate in the present study was found between 4.30 and 62.59 mg/kg in 86.66% of the samples (Table 2). In previous results reported in Turkey, the amounts of residual nitrate were detected between 1.56 - 943.71 mg/kg (Table 3). However, our result was much lower than the result found by some other researchers (Sancak *et al.,* 2008; Sezer *et al.,* 2013; Büyükünal *et al.,* 2016; Benli *et al.,* 2017). The findings of nitrate may result from the use of nitrate in production and the introduction of products in a short period of time without sufficient ripening. In addition, this presence may also be due to the oxidation of nitrite to nitrate and/or from the nitrate which may be found in water and spices used during production (Honikel, 2008).

In the present work, residual nitrite was present in 17.77% of fermented sausage samples between 8.94 - 55.95 mg/kg. Studies in different parts of Turkey revealed variable results ranging from 0.46 to 216.63 mg/kg. Our result was close to the results determined by Sancak *et al.* (2008) and Büyükünal *et al.* (2016), while quite lower than the results found by Sezer *et al.* (2013) and Benli *et al.* (2017) (Table 3).

The presence frequency of residual nitrate was statistically higher in the samples from local butcher shops whereas the presence frequency of residual nitrite was lower in the samples from local butcher shops. Different results may arise from factors such as differences in production practices, the addition of ascorbic acid, pH of the product, storage time, and temperature (EFSA, 2003; Honikel, 2008; Sindelar and Milkowski, 2012).

Conclusion

In the production of traditional fermented sausage, the use of contaminated raw materials, production that is not carried out in a controlled and hygienic manner, and sausages containing high amounts of chemical additives with possible adverse health effects make the final product microbiologically and chemically risky for public health. Therefore, it is important to increase awareness of companies about measures to prevent the microbiological and chemical foodborne exposures from being present in the product. In addition, establishment of systems, where the additives to be used, that can be monitored during the production period is essential. The implementation of effective control measures by competent authorities is also important in order to prevent the risks that may occur in terms of public health.

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