ASSESSMENT OF MICROBIOLOGICAL SAFETY AND QUALITY IN THE MEAT PROCESSING SECTION OF IULS

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Abstract

Preventing meat contamination under production conditions requires appropriate hygiene and food safety measures. These include implementing rigorous hygiene practices, controlling temperature and humidity during the production process, conducting regular inspections of product quality and safety, and using properly sanitized equipment. Contamination can occur due to a variety of factors, including inconsistent hygiene practices, contaminated equipment, and improper handling. Therefore, this study was initiated to assess the microbiological quality of meat from the main steps of the sausage production flow in the meat processing unit of USV Iasi. Meat samples were taken from six production points (whole carcass, meat trimmings, coarsely ground meat, finely minced meat, minced meat and spice mix, sausages before heat treatment) as well as from the finished product after heat treatment. Microbiological analyses were performed on three culture media: Nutrient Agar, Violet Red Bile Dextrose (VRBD) and Rapid E-coli, to identify the total number of aerobic bacteria and fungi, the colonies belonging to the Enterobacteriaceae family, respectively, for direct confirmation of Escherichia coli colonies. The results showed contamination levels below the detection limit mainly for carcass samples and finished product, values for TAB ranging from 1.1 x 10^3 cfu/g for sausages before heat treatment and 2.7 x 10^3 cfu/g for fine minced meat, and values for Enterobacteriaceae ranging from 0.3 x 10^2 cfu/g for meat trimmings and 2.7 x 10^2 cfu/g coarsely ground meat. In this study, all samples collected from the meat processing section exhibited minimal counts of spoilage microorganisms, confirming adherence to hygiene rules and good practices.

Key words: *meat contamination, microbiological analysis, total aerobic bacteria (TAB)*

INTRODUCTION

Meat serves as a primary protein source and a rich provider of essential vitamins for a significant portion of the global population. It plays a crucial role in supporting the growth, repair, and maintenance of body cells, ensuring our everyday vitality. Nevertheless, fresh meat is highly susceptible to contamination, despite its nutritional significance [1].

The meat industry is a major contributor to global food security, with cattle meat alone accounting for 20% of global protein consumption [2]. This has underscored the importance of ensuring the production of safe processed foods through the implementation of global food safety standards. These standards aim to enhance and standardize manufacturing practices, promote good hygiene practices, and establish hazard analysis critical control points [3,4].

Meat is susceptible to contamination at multiple stages of its production, spanning from the primary stages of obtaining to its final preparation for consumption, a process often referred to as „farm-to-fork". Taking into account the susceptibility of meat to contamination and spoilage, contaminated meat represents a significant contributor to foodborne illnesses, contributing to a considerable number of cases and fatalities arising from the ingestion of harmful agents [5]. These foodborne diseases originate from the consumption of food harboring bacteria, toxins, and microbial cells

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produced within the food matrix [6]. A report from the European Food Safety Authority and European Centre for Disease Prevention & Control (EFSA-ECDC) states that, in 2018, 642 cases from the 5079 outbreaks related to food and water were confirmed as „strong evidence" food-related incidents. Among these, approximately 60% (385 outbreaks) were linked to food from animals, with meat and meat products being the most commonly implicated foods [7].

In the meat industry, processing stages are often more susceptible to pathogen contamination compared to production phases. Sources of contamination encompass environmental elements such as soil, faeces, and water. Additionally, insufficiently sanitized surfaces involved in food contact, such as conveyor belts, knives, and slicers, pose a risk, as do non-food contact surfaces like walls, drains, and floors. Unhygienic facility design, unregulated technological flows, a lack of hand sanitation among workers, and transport trailers and crates can also contribute to contamination [8,9].

Moreover, various factors contribute to the presence of harmful microorganisms, including the use of contaminated raw meats, as well as potential contamination from the surrounding environment. To address these concerns and establish effective control measures for ensuring the safety of meat products, this study aimed to assess possible contamination that may occur throughout the pork sausage manufacturing process, from raw material to finished product, in the meat micro-production section of ULS Iasi.

MATERIAL AND METHOD

Microbiological testing holds paramount importance in the food industry, as it plays a pivotal role in ensuring food safety, maintaining quality, and adhering to food regulations and standards. These analyses serve to identify potential risks that could impact consumers and facilitate corrective actions to uphold products of superior microbiological quality.

To achieve our research objective, we collected meat samples from the pork sausage production process within the meat processing section of the University of Life Sciences Iasi. Stringent hygienic conditions were observed during sampling, with the use of sterile instruments and equipment to maintain sample integrity. Meat samples were obtained from seven distinct points along the production process, including whole carcasses, meat trimmings, coarsely ground meat, finely minced meat, minced meat with spice mix, sausages before heat treatment, and sausages after heat treatment.

The collected samples underwent determination of the total aerobic bacteria count and the count of *Enterobacteriaceae* using the method of successive dilutions, following the procedure outlined by Ulea E. & Lipșa F. (2012) [10]. Dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were prepared. Subsequently, 1 ml of each dilution was pipetted onto Petri plates, using three different culture medium —Nutrient Agar, Violet Red Bile Dextrose, and Rapid E. coli—depending on the target microorganism.

To ensure the viability of microorganisms, the culture media were cooled to a temperature below 45°C before inoculation. Cooling was achieved through a water jet, with temperature monitoring using a thermometer. After the introduction of the culture medium, the plates were gently swirled in a circular motion to evenly disperse the inoculum and facilitate uniform colony distribution.

Following the solidification of the medium, the plates underwent incubation, with varying conditions based on the medium used. Nutrient Agar required incubation at 28°C for 24-36 hours to enumerate aerobic bacteria and fungi. Rapid *E. coli*, a chromogenic agar used for direct confirmation of *Escherichia coli* colonies, and VRBD, employed for colonies belonging to the *Enterobacteriaceae* family, underwent incubation at 37°C for 24 hours.

Upon completion of the incubation period, Petri plates were removed and placed in an automatic colony counter, which featured a contrast surface (HD automatic colony counter Scan 1200, Interscience, France).

RESULTS

The results obtained from colony counting on each plate using the three culture media were documented for each dilution, revealing a progressive reduction in colony counts from 10^{-1} to 10^{-3}. Consequently, the results were reported based on the 10^{-1} dilution, which exhibited the highest microbial load among the plates.

Subsequently, the average results obtained for the seven stages within the sausage production process flow, encompassing total aerobic bacteria (Table 1), *Enterobacteriaceae* load (Table 2), and identification of *E. coli*/coliform/non-coliform bacteria (Table 3), were juxtaposed with a reference range stipulated in Commission Regulation (EC) No 1441/2007 concerning process hygiene criteria in the meat industry [11].

The mean total aerobic bacteria (Table 1) showed values below the detectable level for whole carcass samples and for the final product (sausages after the heat treatment).

Table 1 Total aerobic bacterial load (cfu/g) of samples in Nutrient Agar

Samples	Mean (cfu/g x10^3)	Std. dev. (cfu/g)	Range (cfu/g x10^3)	Commission Regulation (EC) No 1441/2007*	
				m	M
Whole carcass	BDL	-	BDL	10^4 cfu/g	10^5 cfu/g
Pork trimmings quality 1	2.4	40.41	2.4 – 2.5	5 x 10^5 cfu/g	5 x 10^6 cfu/g
Coarsely ground meat	2.4	17.06	2.4 – 2.5		
Fine minced meat	2.7	63.52	2.7 – 2.8		
Minced meat and spice mix	1.2	27.30	1.2 – 1.3		
Sausages before heat treatment	1.1	77.69	1.0 – 1.2		
Sausages (final product)	BDL	-	BDL		

**Limits according to Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs; m= medium contamination level; M= maximum contamination limit. BDL: below detectable level*

Table 2 Microbial load (*Enterobacteriaceae*, cfu/g) of samples in VRBD

Samples	Mean (cfu/g x10^2)	Std. dev. (cfu/g)	Range (cfu/g x10^2)	Commission Regulation (EC) No 1441/2007*	
				m	M
Whole carcass	BDL	-	BDL	4 x 10^2 cfu/g	6 x 10^2 cfu/g
Pork trimmings quality 1	0.3	2.08	0.2 – 0.3		
Coarsely ground meat	2.6	69.48	2.0 – 3.4		
Fine minced meat	1.8	14.05	1.6 – 1.9		
Minced meat and spice mix	BDL	-	BDL		
Sausages before heat treatment	BDL	-	BDL		
Sausages (final product)	BDL	-	BDL		

**Limits according to Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs; m= medium contamination level; M= maximum contamination limit. BDL: below detectable level*

The test performed on Rapid E. coli medium (Table 3) confirmed the results previously obtained for the quantification of bacteria of the *Enterobacteriaceae* family, obtaining results above the detection limit only for samples taken from meat trimmings (0.59 x 10^3 cfu/g), ground meat (0.76 x 10^3 cfu/g) and minced meat (6.1 x 10^3 cfu/g).

Table 3 Microbial load (*E. coli* / Coliforms / Non-coliforms, cfu/g) of samples

Samples	Mean (cfu/g x10^3)	Std. dev. (cfu/g)	Range (cfu/g x10^3)	Commission Regulation (EC) No 1441/2007*	
				m	M
Whole carcass	BDL	-	BDL	10^4 cfu/g	10^5 cfu/g
Pork trimmings quality 1	0.59	28.75	0.56 – 0.61		
Coarsely ground meat	0.76	86.56	0.69 – 0.86		
Fine minced meat	0.61	23.52	0.59 – 0.63		
Minced meat and spice mix	BDL	-	BDL		
Sausages before heat treatment	BDL	-	BDL		
Sausages (final product)	BDL	-	BDL		

**Limits according to Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs; m= medium contamination level; M= maximum contamination limit. BDL: below detectable level*

DISCUSSIONS

Meat and meat products, owing to their elevated water content and chemical components, provide an ideal environment for the proliferation of diverse microorganisms, ultimately resulting in spoilage and diminished health safety.

The results for the total aerobiotic bacteria below the detection limit are due, for the carcass sample, to the strict rules imposed on the processing facilities, according to which all carcasses received must be compliant and devoid of any risk to consumers. In the case of sausage samples, the high temperature during heat treatment has the effect of reducing or partially eliminating bacteria, as also stated by different authors [12,13,14].

The highest contamination value recorded was in the sample of finely minced meat, resulting from the cutter mincing, i.e. a mean value of 2.7 x 10^3 cfu/g. The number of bacteria in the minced meat is mainly due to the handling and contact of the meat with the equipment used. In order to keep the microbial load at a low level, it is necessary to operate well-sanitised equipment and to respect the optimal working temperature.

Considering the reference range of 5 x 10^5 – 5 x 10^6 aerobic bacteria stipulated in Commission Regulation (EC) No 1441/2007 of 5 December 2007, all the study results show values below the medium contamination level.

By using the V.R.B.D. culture medium, the aim was to highlight the *Enterobacteriaceae* that have developed as a result of thermostatting (Table 2). The *Enterobacteriaceae* family encompasses a diverse group of bacteria, including pathogenic strains like *Salmonella*, *Escherichia coli*, and *Shigella*, known to pose health risks to humans. It is imperative to employ robust detection and identification methods for these bacterial strains in meat products to proactively mitigate the potential for foodborne illnesses.

Values above the detection limit were found for only three of the seven meat samples analysed, namely for meat trimmings, coarsely ground meat and fine minced meat. The stage in the production flow with the highest value for *Enterobacteriaceae* counts was the coarse mincing at the wolf, 2.6 x 10^2 cfu/g, followed by fine mincing cutter with 1.8 x 10^2 cfu/g and the lowest value of 0.3 x 10^2 cfu/g for meat trimmings. The phenomenon can be attributed to the intrinsic ability of meat to sustain an amplified microbial load during its traversal through meat cutting and processing areas. These operations entail a relatively intensive degree of manipulation and handling of meat, thus significantly heightening the microbial risks.

In addition to the samples where the microbial load was very low, below the detection limit (carcass, finely minced meat and spice mix, sausage before heat treatment and finished product), the other samples analysed were below the reference

range 4 x 10^2 - 6 x 10^2 cfu/g required in Regulation (EC) No 1441/2007.

Monitoring *Enterobacteriaceae* levels in meat is an essential component of quality control measures within the meat industry [15,16]. Reduced levels of *Enterobacteriaceae* serve as an indicator of improved hygiene and sanitary conditions during meat processing and handling. This monitoring helps assess the effectiveness of sanitation protocols and adherence to food safety standards. Additionally, it plays a pivotal role in ensuring that meat products meet the requisite microbiological safety criteria, reducing the risk of foodborne illnesses and safeguarding public health. Continuous vigilance and meticulous monitoring of *Enterobacteriaceae* levels contribute significantly to enhancing the overall quality and safety of meat products, which is paramount in the food industry.

Similarly, for the identification of *E. coli* / Coliforms / Non-coliforms (Table 3), a higher microbial load is observed in the cutting processing stages. The primary mechanism facilitating this risk escalation is the potential for microbial cross-contamination, which occurs through various vectors, including manual contact via hands, as well as through utensils such as knives, saws, and conveyors. Furthermore, the transfer of bacteria from the external surfaces of meat to its internal portions exacerbates this issue. Additionally, human-related factors, including personnel surfaces such as hands and clothing, as well as the contact surfaces of meat processing equipment, like saws and mincers, knives, and cutting boards, consistently contribute to the contamination of meat [17,18,19].

CONCLUSIONS

The contamination of meat can be attributed to several major factors, including low-level awareness of hygienic practices, disruptions in the cold chain, or inadequate sanitation of processing units.

The outcomes of our research show that all samples fall within the limits set out in Commission Regulation (EC) No 1441/2007 of 5 December 2007 on microbiological criteria for foodstuffs, with the highest microbial load observed in the meat sample collected from the cutting processing stages (cutting, grounding and mincing). These results derive from and confirm at the same time a thorough maintenance of good hygienic practices, hygiene of personnel and equipment used, properly conducted thermal processing and hot smoking, effective temperature control throughout food production, as well as the use of high quality raw materials.

REFERENCES

1. Zerabruk, K; Retta, N; Muleta, D; Tefera, AT Assessment of microbiological safety and quality of minced meat and meat contact surfaces in selected butcher shops of Addis Ababa, Ethiopia. *Journal of Food Quality* **2019**, *2019*, 1-9. https://doi.org/10.1155/2019/3902690
2. Mosnier, C; Jarousse, A; Madrange, P; Balouzat, J; Guillier, M; Pirlo, G; Mertens, A; Oriordan, E; Pahmeyer, C; Hennart, S; Legein, L; Crosson, P; Kearney, M; Dimon, P; Bertozzi, C; Reding, E; Iacurto, M; Breen, J; Carè, S; . . . Veysset, P Evaluation of the contribution of 16 European beef production systems to food security. *Agricultural Systems* **2021**, *190*, 103088. https://doi.org/10.1016/j.agsy.2021.103088
3. Lo, MY; Ngan, WY; Tsun, SM, Hsing, H; Lau, KT; Hung, HP; Chan, SL; Lai, YY; Yao, Y; Pu, Y; Habimana, O A Field Study Into Hong Kong's Wet Markets: Raised Questions Into the Hygienic Maintenance of Meat Contact Surfaces and the Dissemination of Microorganisms Associated With Nosocomial Infections. *Frontiers in Microbiology* **2019**, *10*, 455989. https://doi.org/10.3389/fmicb.2019.02618
4. Ripolles-Avila, C; Hascoët, A; Martínez-Suárez, J; Capita, R; Rodríguez-Jerez, J Evaluation of the microbiological contamination of food processing environments through implementing surface sensors in an iberian pork processing plant: An approach towards the control of *Listeria monocytogenes*. *Food Control* **2019**, *99*, 40-47. https://doi.org/10.1016/j.foodcont.2018.12.013

5. WHO, 2007 – Food Safety and Food Borne Illness, World Health Organization, Geneva, Switzerland, Fact sheet No. 237.
6. Bersisa, A; Tulu, D; Negera, C Investigation of Bacteriological Quality of Meat from Abattoir and Butcher Shops in Bishoftu, Central Ethiopia. *International Journal of Microbiology* **2019**, *2019*, 1–8. https://doi.org/10.1155/2019/6416803
7. Nielsen, B; Colle, MJ; Ünlü, G Meat safety and quality: A biological approach. *International Journal of Food Science & Technology* **2020**, *56* (1), 39-51. https://doi.org/10.1111/ijfs.14602
8. Das, AK; Nanda, PK; Das, A; Biswas, S Hazards and safety issues of meat and meat products. In: Food safety and human health **2019** (pp. 145-168), Academic Press.
9. Thakali, A; MacRae, JD A review of chemical and microbial contamination in food: What are the threats to a circular food system? *Environmental Research* **2021**, *194*, 110635. https://doi.org/10.1016/j.envres.2020.110635
10. Ulea, E; Lipșa, FD Îndrumător practic de microbiologie. Editura Ion Ionescu de la Brad **2012**, Iași.
11. Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (available online at: https://eur-lex.europa.eu/eli/reg/2007/1441/oj)
12. Fudali, A; Chełmecka, I; Salejda, AM; Krasnowska, G Microbiological Safety and Organoleptic Quality of Homogenized Sausages Manufactured with Commercial Functional Additives. *Applied Sciences* **2020**, *11* (24), 11662. https://doi.org/10.3390/app112411662
13. Carballo, J Sausages: Nutrition, Safety, Processing and Quality Improvement. *Foods* **2021**, *10* (4), 890. https://doi.org/10.3390/foods10040890
14. Ercoşkun, H The effects of fermentation time on heat treated sucuk (turkish-style dry-fermented sausage). *Journal of Food Safety & Food Quality* **2021**, *72* (5).
15. Halkman, HBD; Halkman, AK Indicator organisms, in: C.A. Batt, M.L. Tortorello (Eds.), Encyclopedia of food microbiology (second edition) Academic Press **2014**, pp. 358-363.
16. Mladenović, KG; Grujović, MŽ; Kiš, M; Furmeg, S; Tkalec, VJ Stefanović, OD; Kocić-Tanackov, SD *Enterobacteriaceae* in food safety with an emphasis on raw milk and meat. *Applied Microbiology and Biotechnology* **2021**, *105,* 8615–8627. https://doi.org/10.1007/s00253-021-11655-7
17. Nortje, GL; Nel, L; Jordaan, E; Naudé, RT; Holzapfe,l WH; Grimbeek, RJ A microbiological survey of fresh meat in the supermarket trade. Part 1: Carcasses and contact surfaces. *Meat* Science 1989, *25*(2), 81-97. https://doi.org/10.1016/0309-1740(89)90024-7
18. Nørrung, B; Buncic, S Microbial safety of meat in the European Union. Meat science **2008**, *78* (1-2), 14-24. https://doi.org/10.1016/j.meatsci.2007.07.032
19. Tomasevic, I; Kuzmanović, J; Anđelković, A; Saračević, M; Stojanović, MM; Djekic, I The effects of mandatory HACCP implementation on microbiological indicators of process hygiene in meat processing and retail establishments in Serbia. *Meat Science* **2016**, *114*, 54-57. https://doi.org/10.1016/j.meatsci.2015.12.008