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MICROBIOLOGICAL METHODS OF IMPROVING THE SAFETY AND SECURITY OF MEAT AND MEAT PRODUCTS

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Abstract

The consumer trend towards wholesome, minimally processed meat products has put enormous pressure on processors to certify the safety of meat and meat products without sacrificing product quality and meeting consumer demand. This has caused difficulties in the creation and implementation of new technological advances, since the use of more modern innovations can influence consumer decisions and their assessment of the quality of meat and meat products. New cooking technologies in the meat industry require microbiological approval before being named industrially viable alternatives and authorizing infrastructural changes. Food spoilage by pathogens causes a variety of foodborne illnesses that can even lead to death, resulting in economic losses. This article presents literature data on the impact of microorganisms on the quality of meat and meat products, methods of microbiological control.

Keywords: Microbiological spoilage, meat and meat products, lactic acid bacteria, pathogenic microorganisms.

Food safety is a global problem, a priority for both research and practical activities in the food industry. Food safety systems are much better in developed countries and worse in developing ones. This explains the large number of food crises in these countries, especially in Asian countries.

Meat continues to play an important role in the human diet as a good source of high quality protein as well as healthy fatty acids and a variety of micronutrients for optimal health. [1]. Meat contains a protein of high biological value with all the essential amino acids needed by adults and children. Meat contains an average of 20-24 g of protein per 100 g (raw), making it one of the main sources of protein[2] . Meat also contains a wide range of bioavailable micronutrients, such as iron, which are essential for overall health and well-being[3-4] .

Food spoilage occurs as a result of microbiological, chemical, or physical changes that render a food product unacceptable to the consumer. Microbiological spoilage of food is caused by the growth of microorganisms that produce enzymes that produce unwanted by-products in food. Meat is one of the most perishable foodstuffs and is widely associated with foodborne illness, as extensive outbreaks have been associated with the consumption of contaminated meat [5].

Animal products must be controlled to ensure that people can obtain meat suitable for consumption. Meat can pose biological, physical and chemical hazards that can occur at any stage of the supply chain, from slaughter to serving. Pathogenic microorganisms are commonly found in the digestive tract of healthy cattle. These microorganisms can also be found on the skins of live animals contaminated with faeces, which can then be transferred to the surface of previously sterile meat during slaughter, especially when it is carried out on the floor in the absence of a carcass suspension system in sloppy evisceration, in which intestinal contents are distributed over the surface. meat. Carcasses of cattle can be contaminated during the slaughter process by contact with the skin and hair of the animal, limbs, blood, stomach contents, intestines, bile and other secretions, premises, equipment, as well as the hands and clothing of the worker [6]. For these reasons, it is necessary to pay special attention to hygiene and sanitation in the process of slaughtering livestock [7].

Providing a quality product and increasing the shelf life of food is one of the important and main directions of the food industry. The use of various synthetic dyes, flavors, preservatives to extend the shelf life of meat and meat products, leads to a threat to the health of consumers. Even with the use of modern conservation methods, spoilage remains an unresolved problem[8].

Food, which by its nature is raw, remains protected from attack by microorganisms due to specific structures such as skins, shells, bran, etc., which are not degradable. Meat and fish products are more susceptible to spoilage when raw due to the favorable conditions for microbial spoilage. Surface contamination usually occurs at an earlier stage in the processing of raw meat and fish. When the protective layer or packaging is removed, food products tend to be more vulnerable to spoilage, which also accelerates the spread of microorganisms once the processing step begins[9].

The spoilage of raw meat is mainly due to the undesirable development of microbes in the meat during storage. The type of bacteria and their number depend on the initial contamination of the meat and on the specific storage conditions, which can influence the development of various microbial populations associated with spoilage, thus affecting the type and speed of the spoilage process [10].

If the regimes and terms of refrigeration storage of meat are violated, as a result of the multiplication of microorganisms, its quality may change, which leads to product spoilage. There are several types of spoilage of chilled, frozen and thawed meat: mucus, rotting, sour (acidic) fermentation, pigmentation (appearance of age spots), glow and mold.

The main causative agents of mucus are aerobic psychrophilic gram-negative bacteria, most often of the genus *Pseudomonas*, as well as aerobic yeasts. When meat is stored at -5°C, micrococci, streptococci, actinomycetes, some putrefactive bacteria and other mesophilic microorganisms multiply, having the lowest minimum growth temperature. Rotten meat is caused by various aerobic and facultative anaerobic non-spore-forming bacteria, as well as spore-forming aerobic and anaerobic bacteria, most often of the genus *Pseudomonas*. At elevated storage temperatures, meat rotting is caused by mesophilic putrefactive microorganisms: non-sporeforming bacteria Proteus vulgaris, *Serratia marcescens, Bacillus subtilis, Bacillus. mesentericus, Bacillus mycoides*; other aerobic bacilli; anaerobic *Clostridium sporogenes, Clostridium putrificus and Clostridium perfringens*. The causative agents of acid fermentation of meat are psychrophilic lactobacilli, micobacteria and yeast, which are able to develop in the depths of muscle tissue, where a low oxygen concentration is created. The causative agents of pigmentation are *Pseudomonas fluorescens, Pseudomonas aeruginosa, Serratia* marcescens and other aerobic bacteria, various sarcins, pigment yeasts, most often of the genus *Torula*. Frozen meat molds are most often caused by molds of the genera *Thamnidium, Rhizopus,* and *Cladosporium,* which have the lowest minimum growth temperature [11]. Meat spoilage microorganisms grow under certain conditions (Table 1).

Table 1 - Temperature values that determine the possibility of growth of microorganisms typical for meat products and the consequences caused by their development

	$\overline{2}$	3	4
			slightly concave, matte colonies. The edge
			is wavy. The cells are large $1 \times 3-4$
			microns, the endospores are centrally
			located, do not exceed the cell size. The
			flagella are arranged peritrichially. gram-
			positive, spore-forming
Staph.aureus	\geq 7	Spoilage and	Spherical, gram-positive,
		poisoning	large 1×3 -4 microns
			Gram-negative rods measuring 0.4 -0.6-2.6
Escherichia	\geq 7	Spoilage and	microns, mobile due to peritrichially
		poisoning	arranged flagella, form colonies in R- and
			S-forms.
Salmonella	≥ 5	Spoilage and poisoning	Movable (thanks to flagella – peritrichs),
			asporogenic gram-negative straight rods
			$(0.5-1x1-3$ microns) with rounded ends
			gram-positive anaerobes are non-spore-
Lactobacillus	≥ 0	Spoilage	forming, have the correct shape of a long
			"stick", sometimes coccoid, are arranged
			in short chains or singly.
			грамотрицательные бактерии. Размеры
Pseudomonas	≥ -5	Spoilage	клеток 0,5-1х1,5-4 мкм
			sizes from 2.5 to 10 micrometers across
Yeasts	≤ -5	Spoilage	and from 4 to 20 microns in length. The
			shape of the cells is elongated, oval,
			ellipsoid, lemon-shaped or spherical
Mold fungi	≤ -5	Spoilage and poisoning	they form velvety, powdery, felt, spider-
			like, mossy deposits of green, white,
			black, yellow and other colors of various
			shades. Gifs can be short or long. Their
			thickness ranges from 1 to 15 microns,
			length — from 2065 to 50 microns or
			more.

Table 1 continued

Losses associated with microbiological spoilage of meat represent a serious economic problem on a global scale. Today, in the meat industry, microbial spoilage of meat during storage causes significant financial losses to the industry. According to the Food and Agriculture Organization (FAO), a third of all food produced in the world is wasted every year - about 1.3 billion tons. The total volume of lost products for developed and developing countries is comparable, however, in developed countries, losses of meat products predominate, reaching 67% of the global losses of meat processing industries [12-14].

Meat and meat products provide an excellent environment for the growth of a variety of microflora (bacteria, yeasts and molds), some of which are pathogenic [15]. The dominant spoilage microorganisms are gram-negative aerobic bacilli *(Escherichia coli, genus Pseudomonas*), coccal bacteria *(Staphylococcus epidermidis, Staphylococcus aureus*), facultative anaerobes (*Aeromonas hydrophila), Salmonella spp.* [16]. The qualitative composition of microorganisms and their initial concentration are not constant values, but depend on a variety of factors, including the type and initial concentration of microorganisms, the nature of the interaction of populations, temperature and time factors of storage of raw materials, the presence of conditions conducive to microbial contamination [17] .

The growth of foodborne pathogens such as *Salmonella* and toxin-producing strains of *E. coli, Listeria monocytogenes, Clostridium perfringens,* and *S. aureus* are of greatest concern in the production of meat and poultry products [18–20]. These bacteria are the most common cause of foodborne illness. Listeria monocytogenes is the causative agent of listeriosis in humans and animals. Currently, this disease is considered one of the most significant foodborne infections in the world. The main transmission factors for listeriosis are milk and dairy products, animal and poultry meat, vegetables and seafood [21].

The most significant Gram-positive microorganism that has received attention due to associated nosocomial and community-acquired infections is *S. aureus* [22-24]. In addition to poultry meat, *S. aureus*, as well as methicillin-resistant *S. aureus*, is found in the meat of pigs [25] and cattle [26]. This bacterium multiplies rapidly at room temperature, producing toxins that cause food poisoning [27].

In public health and economics, in both developed and developing countries, *Salmonella spp.* continue to cause serious problems *Salmonella enteritidis* and *Salmonella typhimurium* are the most frequently reported serotypes causing human salmonellosis in both the EU and the USA [28-32]. This highlights the need to improve the prevention and control of *Salmonella spp.* in food products. *Salmonella* are Gram-negative organisms of the *Enterobacteriaceae* family, which may be indistinguishable from *E. coli* under a microscope or when cultured on conventional nonselective nutrient[33]. Despite significant changes that have been made over time to the taxonomy and nomenclature of the genus *Salmonella*, it is now well established that the genus consists of only two genomic species, i.e. *Salmonella enterica* and *Salmonella bongori. Salmonella enterica* strains are responsible for 99% of *Salmonella* infections in humans and warm-blooded animals and are usually transmitted through ingestion of contaminated food or water; on the other hand, strains of the other five subspecies, as well as *S. bongori*, are commonly isolated from foodborne pathogens related to meat safety. *S. enterica*, due to its high association with food animals such as poultry, cattle and pigs, the pathogen is commonly associated with the raw meat of these and other farm animals [34].

Staphylococcal infection is the most economically important foodborne disease [35]. It causes gastrointestinal illness due to a wide range of toxins [36], including staphylococcal enterotoxins that cause vomiting and diarrhea within 2–6 hours of ingestion of contaminated food [37–39].

Psychrotrophic *Pseudomonas* species are the key microorganisms causing spoilage of chilled meat during aerobic storage. *Pseudomonas* are highly resilient and able to withstand stressful environmental conditions that would otherwise inhibit the growth of other spoilage organisms [40].

E.coli is a bacterial species of the *Enterobacteriaceae* family that includes both pathogenic and non-pathogenic strains, the latter being the majority of the facultative microflora found in the gastrointestinal tract of most vertebrates [41]. With respect to pathogenic E. coli, there are six foodborne disease-associated pathotypes responsible for gastrointestinal infections recognized as the causative agent of serious illness and death during foodborne disease outbreaks worldwide[42]. In 1982, *E. coli* was first associated with an epidemic of foodborne illness associated with the consumption of improperly cooked hamburgers in the United States, and a new foodborne zoonosis was identified [43].

Most *Clostridium* are saprophytes, four species have been identified as human pathogens, namely *Cl. perfringens, Clostridium botulinum, Clostridium difficile* and *Clostridium tetani.* Among these species, *Cl. perfringens* and *Cl. botulinum* are well-established foodborne pathogens. Food poisoning usually results from ingestion of high concentrations (>10⁶) of viable *Cl vegetative* cells. perfringens, which are commonly found in processed foods. Symptoms of foodborne illness caused by *Cl. perfringens* include acute abdominal pain and diarrhea, while the pathogen's enterotoxin is also thought to play a role in the etiology of SIDS . For meat, the organism may either be initially present in muscle tissue or be introduced via faecal contamination into carcasses at slaughter or into meat products during subsequent processing [44].

The genus *Yersinia* consists of at least 12 species, among which three species are considered pathogens for humans: *Yersinia enterocolitica, Yersinia pseudotuberculosis,* and *Yersinia pestis* [45]. A foodborne illness caused by *Yersinia spp.* called yersiniosis is caused by *Y. enterocolitica* or *Y. pseudotuberculosis Yersinia spp.* are gram-negative, non-spore-forming rods or coccobacilli that are facultative anaerobes and are able to grow at refrigeration temperatures. Although the optimum growth temperature for *Y. enterocolitica* is approximately 30°C, it can support growth at temperatures as low as 0° C. Animals have traditionally been considered the main reservoir of pathogenic *Yersinia spp*., with slaughtered pigs being the single most important source of *Y. enterocolitica*. More specifically, the *Y. enterocolitica* serotype appears to be distributed worldwide and is the most commonly isolated foodborne pathogen serotype. [46].

Heterofermentative lactic acid bacteria like *Lactobacillus spp.,* mainly *L. curvatus* and *L. sayi, Leuconostoc spp., Carnobacterium spp.* [47] most involved in meat spoilage. As a result of their metabolism, heterofermentative lactic acid bacteria produce significant amounts of undesirable catabolites such as $CO₂$, ethanol, acetic acid, butanoic acid, and acetoin, with subsequent unpleasant odors and visual effects, such as the formation of stringy mucus and discoloration of meat [48].

The perishable nature of meat requires the constant development and application of new and innovative technologies to kill and/or prevent the growth of pathogens and spoilage microorganisms. Numerous studies show the ongoing change and increase in resistance of pathogenic microorganisms to antimicrobials and conventional food protection methods such as: low pH, heat treatment, drying, temperature reduction and/or minimization of water activity, use of chemicals, preservatives.

The food industry uses many meat preservation methods to prevent and control foodborne pathogens and spoilage microorganisms in fresh meat products, such as vacuum packaging and modified atmosphere packaging [49], packaging films immobilized with antimicrobial agents [50- 54], antimicrobial sachets and absorbent pads [55], edible coatings with inherent antimicrobial properties [56].

Fermentation of foods with lactic acid bacteria is a food preservation method that has gained prominence in recent decades due to the ability of lactic acid bacteria to produce bacteriocins that can replace chemical preservatives in the food industry. [57,58] To date, various strains of lactic acid bacteria producing bacteriocin have been characterized with promising results as a biopreservative in various industrial applications [59-61].

Strains of *Lactobacillus casei* showed potential activity against enteropathogenic *Escherichia coli* and *Salmonella* species [62] screened lactic acid from dairy, meat products and agro-industrial waste and isolated a strain of lactic acid bacteria containing a broad-spectrum antimicrobial compound and inhibiting ten indicator gram-positive and gram-negative strains . Probiotic bacteria isolated from various brands of traditional yoghurts in Egypt exhibited antimicrobial activity at a concentration of 10^9 CFU/g in vitro against test indicator pathogens [63]. Strains of *Lactobacterium sakei, Leuconostoc carnosum*, producing bacteriocins (sacacins and leukocins, respectively), inhibit the activity of pathogenic bacteria, in particular bacteria of the genus *Salmonella and L. monocytogenes,* in meat and meat products.[64]

The lactic acid bacteria *Lactobacillus, Lactobacillus plantarum* and *Lactobacillus paraplantarum* isolated from batter (fermented Indian soft rice cakes) have demonstrated antagonistic activity against gram-positive and gram-negative foodborne pathogens such as *S. aureus, E. coli, S. enterica subsp.* [65]. Typhi, In addition, gastro- and bile-resistant lactic acid bacteria and bifidobacteria *Lactobacillus rhamnosus, L. caasei, L. plantarum and Bifodobacterium longum and B. bifidum*, which have been isolated from stool healthy child showed high antagonistic activity against various foodborne pathogens.

Bacteria of the genus *Propionibacterium* have found wide use in cheese making as a cheese microflora (together with lactic acid bacteria, which favors the environment for *Propionibacterium* strains) used in the production of hard rennet cheese. The role of these bacteria in the production of cheese is based on the fermentation of lactates to propionic and acetic acids, which give the final product a specific flavor; they also serve as natural preservatives [66]. Starter cultures consisting of propionic acid bacteria and lactic acid bacteria *(Lactobacillus plantarum, Lactobacillus acidophilus, Penicillium jensenii and Penicillium acidipropionici)* are used in the

production of fermented products. Their combination increases the speed of the fermentation process and protects the final product from mold and rot, in addition to the fact that pickles obtained in this way are enriched with vitamin B12 and have better taste and dietary properties. Studies have been published regarding the use of *P. freudenreichii subsp. shermani* as a health supplement in feta type cheese in 2017[67].

The results of numerous studies suggest that isolates of lactic acid and propionic acid bacteria are possible candidates for the preparation of industrial starters useful for the production of safe and bioprotective products, which, in turn, can be suitable suppliers of probiotic cultures.

Conclusion

Meat safety is one of the most important current and future societal issues, and in order to reduce the burden of foodborne disease, the food industry and public health authorities need to successfully address various challenges. To achieve this goal, it is essential to develop and implement integrated approaches that cover the entire food supply chain, from slaughter to finished products. Since bacterial pathogens, including emerging or evolving pathogens, represent the most serious meat safety problems, more complete and reliable information about the composition of microbial communities and the dynamic processes of their metabolism is needed. By identifying specific interactions between different spoilage phenotypes in microbiological contamination, we could achieve controlled product quality in the production, transportation, marketing and storage of meat.

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