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KNOWLEDGE OF THE FACTORS AFFECTING THE STORAGE LIFE OF RAW MEAT IS THE KEY TO THE RATIONAL USE OF PRODUCTION RESOURCES

A. Lohinova, PhD student
L. Arsenyeva, Doctor of Technical Sciences,
Department of Foodstuff Expertise
National University of Food Technologies
68, Volodymyrska St., Kyiv, Ukraine,01033

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Correspondence:

A. Lohinova
*E-mail:*anna.ol.logi@gmail.com

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Abstract. This work is a review, which presents, generalises, and systematises the information collected by other leading experts from around the world on storage and especially on spoilage of various types of meat. The paper considers the main physical factors of spoilage of meat-based food products, sources of pollution at all stages of the meat production chain, from the arrival of raw materials up to packaging in various consumer containers and storage at different temperatures. The main representatives of foreign microflora (bacteria, moulds, yeasts, etc.) causing spoilage of raw meat have been briefly described, their taxonomic diversity has been analysed, and characteristic diagnostic signs of meat spoilage by microorganisms of certain groups have been found out. Besides, the paper considers and describes the internal and external factors determining the growth, development, and reproduction of microflora, especially those affecting the rate and intensity of raw meat spoilage, and substantiates their interrelationship and mutual influence. Certain regularities have been established in how these factors affect the vital activity of spoilage microflora, and it has been shown that changing their numerical values allows controlling microorganisms' growth, development, and reproduction, thus extending the shelf life of a product. For example, one of the most common physicochemical indicators of meat, which affects its processing method or shelf life, is active acidity. It is a well-known fact that with its increase, the shelf life of meat products decreases. Such an indicator as water activity is an important physical parameter of meat quality and safety, as it is one of the decisive factors of the growth and development of microorganisms. In our opinion, this is one of the key parameters affecting the storage life and stability of meat products. So adjusting its numerical value in various ways will make it possible to improve the existing technologies of storing meat products, and may allow developing and implementing some new ones.

Key words: microbial spoilage of meat, foreign microflora, water activity, perishable product, bacteria, mould fungi, yeast, chicken meat

Introduction. Formulation of the problem

The food loss in the world is a constantly growing problem. As is known from the data of the international congress Safe Food, which took place in the international industrial fair Interpack from 12 May to 18 May 2011 under the auspices of the Food and Agriculture Organisation of the United Nations, it was established that about 1.3 billion of all food products (about a third) is lost. This means that not only food products are lost, but also the resources necessary for their production [1,2]. It is also known that these losses occur throughout the entire food production chain. For industrialised countries, they amount to about 95–115 kg/year per person, being 6–11 kg/year for poor countries. However, this study was based on a large number of estimated and assumed losses, which made it difficult to verify the data and assumptions in that paper.

The constant problem of food products lost at various stages of their life cycle forces scientists all over the world to research this issue and look for new ways to solve the problem [3,4]. Thus, reducing food losses will help not only to decrease the hunger-caused mortality rate [5,6], but also to reduce the environmental pollution and to use rationally available natural resources [7,8].

Product losses during technological processing and storage are a problem of the meat processing industry too, since meat and its processing products are perishable products due to their high water content and a significant proportion of proteins and fats, which are a good culture medium for foreign microflora [9,10]. That is why solving problems of product spoilage, including studying the factors that affect the rate of spoilage of food products, never ceases to be a topical issue.

According to the data of world organisations, it is predicted that by 2100 there will be about 10.9 billion people in the world [11]. Consequently, the growing number of people will make it necessary to produce more food. One of the conditions for maintaining people's excellent health is eating high-quality and safe products constantly. However, in the modern world, even with the constant advance in science and technology, it is still not possible to produce such products on a global scale. This is due to the fact that the quality of food products during technological processing deteriorates in comparison with their initial characteristics, and thus, the process of their spoilage accelerates [12].

Food spoilage leads to large economic losses for both farmers (producers) and consumers (buyers). A number of factors affect the rate of food spoilage: storage temperature, access of water, pH, initial microbial contamination, the presence of microorganisms that actually cause spoilage, the method of processing the product (sterilisation/pasteurisation, freezing/chilling, smoking, etc.), etc. Spoilage can be both visible (change in the appearance, taste, smell, etc.) and invisible to the eye (usually due to microbial spoilage) [13-15].

There are 3 main types of food spoilage, which are classified according to the basic principles underlying their occurrence – physical, chemical, and microbial. There is some correlation between them, and, as a rule, deterioration caused by a certain type of food spoilage processes can facilitate the development of another type of deterioration.

Food spoilage occurs when the microbiological, chemical, or physical changes taking place in a product make it unfit for consumption. Microbial spoilage of food is caused by the growth of microorganisms producing enzymes, which in turn lead to the appearance of undesirable by-products of their vital activity in food. Chemical spoilage of food occurs when different food components react with each other or with some added component that changes the sensory characteristics of the product. Oxidation, enzymatic and non-enzymatic browning are examples of this process. Physical spoilage of food occurs when high-moisture foods are excessively dehydrated or when dried foods absorb too much moisture, etc. [16,17].

In this paper, we will review in detail the microbial spoilage of meat. This food product is one of the most perishable, because raw meat itself is an excellent culture medium for the growth and development of foreign microflora typically responsible for the spoilage of this product.

The purpose of this paper is to generalise and structure the known scientific data on the spoilage factors of meat and its processing products. Knowledge of the mechanisms of food spoilage will help to improve the existing and develop new reliable technologies in the future to avoid food spoilage and reduce the amount of possible waste.

To achieve the purpose, the following **objectives were set:**

– to consider the general characteristics of food spoilage;

– to characterise microorganisms that cause spoilage of raw meat;

– to describe the internal and external factors that cause raw meat spoilage, and to prove their relationship with the growth of microorganisms.

Analysis of recent research and publications

Nowadays, at the modern stage of technological development of the food industry, it is still important to solve the problem of how to improve the processing and storage of meat products and use rationally materials and human resources in their manufacture. Despite the progress in related industries, which ensure the improvement of the quality of food products, their processing technologies, methods and means of their packaging, transportation, and storage, the problem of extending their shelf life still exists.

A significant part of food manufacture is the meat industry. It is closely related to the agro industrial complex, which is a direct supplier of meat raw materials. Raw meat, including chicken, consists of water (71–76%), proteins (20–22%), and fats (3–8%), and the rest of its components are carbohydrates and other soluble organic and inorganic non-protein substances, as well as minor components – vitamins, colourants, flavours, and aromatic substances [13,18]. At the first stage of stockpiling raw materials, the muscle tissues of healthy animals and poultry contain no spoilage-causing microorganisms but are infected with them as early as during slaughter [13,19-21].

Studies that have gained popularity in the recent decades concern the prevalence, general characteristics, evolution, and, most importantly, interaction of various microorganisms during meat processing and storage [13,22-23]. Also, these studies have shown that only a small part of the wide variety of microbial contaminants in fresh meat develops and dominates the spoilage of the product. The presence or predominance of this or that microbial contaminant in meat depends on a number of factors. They include the composition and types of microorganisms that prevail, pH, water activity, the composition and consistency of raw or processed meat, the method of processing the product, the storage temperature, and the composition of the protective atmosphere in the packaging [13-16,24-29].

Contamination of meat occurs at the stage of slaughter, when the carcass is contaminated with skin from the animal (including fur, feathers, etc.), faeces, intestinal contents, water, through the contact with the personnel, the slaughtering equipment, and even the premises where the slaughter takes place. The initial microbial contamination at the contamination stage can be 10^2 to 10^4 CFU/cm^2 [30]. The main spoilage bacteria found in raw meat are *Acinetobacter*/*Moraxella*, *Psychrobacter*, *Pseudomonas*, *Aeromonas*, *Shewanella putrefaciens*, *Brochothrix thermosphacta*, *Flavobacterium*, *Staphylococcus*, *Micrococcus*, *Enterobacteriaceae*, and lactic acid bacteria of the genera *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, and *Weisella* [13,31].Scientists have established that only 10% of these bacteria present at the initial stage of contamination are psychrotolerant and can reproduce at cold temperatures of cold storage and thus cause meat spoilage [32].

At slaughterhouses, microbial cross-contamination often occurs due to poor sanitary conditions [33]. This can also be due to bacteria's ability to stick to the surfaces of the room and form a film. Both during the pre-slaughter rest of animals and during the processing of carcasses, various bacteria can infect not only carcasses, but also the processing equipment. Faecal coliform bacteria include *Enterobacter*, *Citrobacter*, and *Klebsiella.* The paper [34] indicated that the main source of microbial contamination was slaughterhouse workers. This was confirmed by isolating the same strains of *S. aureus* both from the workers' hands and from the surface of beef carcasses and ground beef. It is also known that the key source of contamination of raw meat is the entrails of animals, including the intestine: it may be damaged when removed, and its contents may get on the surface of the meat. In poultry meat, another source of contamination, besides the above ones, is feathers, which can settle on different parts of the carcass during gutting and thus contaminate the meat.

Carcasses can be contaminated not only by bacteria but by fungi and yeast too. The main sources of contamination of meat with fungi and yeast include air, sewage water, working surfaces and walls of slaughterhouses, equipment and workers of the enterprise [35,36].

In industry, besides domestic animals, the meat of wild animals (game meat) is also used for food. Mostly, this meat is contaminated by intestinal or skin bacteria during slaughter [37]. For example, when the intestine is removed from a deer with only a few hours' delay, this increases the probability that the intestine will be destroyed in the course of removal, since during this delay, it becomes several times bigger in volume [38].

At the stage of transportation of carcasses to a meat processing plant, they can also become contaminated by foreign microflora. This is explained by non-compliance with sanitary regulations at slaughterhouses and in transportation, conditions of loading places, temperature differences during loading/unloading, etc. [39].

Usually, a food product is considered spoilt if it becomes unfit for consumption. Spoilage causes a number of food security problems, when a product can become dangerous for the consumer: result in food poisoning or even death.

Spoilage of meat is caused by a wide variety of microorganisms: bacteria (gram-positive, gram-negative, clostridia, pseudomonads, lactic acid bacteria, etc.), as well as yeast and fungi.

Poultry meat under cold storage conditions is also susceptible to spoilage, since the spoilage process occurs at low temperatures too and is mostly caused by psychrotrophic bacteria (those that can grow and reproduce at low temperatures). Thus, according to [40,41], the specific bacteria that cause spoilage of chilled poultry meat are *Pseudomonas*, *Enterobacteriaceae*, lactic acid bacteria, and *Brochothrix thermosphacta*.

Spoilage of lamb occurs under the influence of such bacteria as *Pseudomonas spp.*, *Shewanella putrefaciens*, *Brochothrix thermosphacta*, psychrotrophic *Enterobacteriaceae*, lactic acid bacteria, and *Clostridium spp.*[42]. Spoilage bacteria characteristic of beef are *Carnobacterium*, *Brochothrix*, *Leuconostoc*, and *Lactococcus* [43]. It is known that the main type of bacteria characteristic of low-quality pork is *Rahnella aquatilis* [44]. In the publication [45], it is stated that at the end of the storage period in French fresh pork sausages, a number of bacteria were revealed: *Brochothrix*, *Pseudomonas*, *Leuconostoc*, and *Lactobacillus*, with the predominance of lactic acid bacteria.

Due to the research [46,47], it was established that *Pseudomonas spp.*, *Brochothrix thermosphacta*, lactic acid bacteria, and yeast were the predominant spoilage bacteria in rabbit meat, because they reproduced faster than other microorganisms and remained until the end of the shelf life. Spoilage of venison is caused by *Clostridium gasigenes* [48], while spoilage of wild boar meat is often caused by members of the *Enterobacteriaceae family* [49]. It is interesting that for chilled vacuum-packed ostrich meat, typical spoilage bacteria are *Pseudomonas* (6.05 log CFU/g), *Enterobacteriaceae* (5.29 log CFU/g), enterococci (0.86 log CFU/g), lactic acid bacteria (6.86 log CFU/g), yeast and mould (4.90 log CFU/g) [50].

For vacuum-packed lamb stored at 1–2 °C for 8 weeks, typical spoilage bacteria were *Clostridium spp.*, which also caused an unpleasant smell [51]. It was established that for beef stored under aerobic conditions at 5 °C, the predominant spoilage bacteria were *B. thermosphacta*, *Pseudomonas*, *Enterobacteriaceae*, and lactic acid bacteria. *B. thermosphacta* was the predominant species in spoilt meat [30].

Vacuum-packed lamb meat stored at 8 °C spoil after 13–16 days, while meat stored at 1.2 °C only after 124 days [52].Specific microorganisms that make meat spoil when it is stored at -1.2 °Care *Carnobacterium*, *Yersinia*, and *Clostridium*, and for storage at 8 °C, these are *Hafnia*, *Lactococcus*, and *Providencia*.

Beef stored in a modified atmosphere at 6°C begins spoiling on days 10–12, the main spoilage microorganisms being*Carnobacterium*, *Brochothrix*, *Leuconostoc*, and *Lactococcus* [43]. In the paper [53], it is said that the deterioration of poultry meat, which was stored in modified atmosphere packaging, occurred after 6–7 days at 10 °C and after 9–10 days at 4°C.

Scientists were able to isolate spoilage microorganisms from meat stored at 4 and 10 °C in a modified atmosphere consisting of 80% O_2 and 20% CO_2.These microorganisms were *Brochothrix thermosphacta*, *Carnobacterium*, and *Pseudomonas*. Also, in poultry meat packed in a modified atmosphere containing 65% N_2 and 35% CO_2, the scientists detected the specific spoilage microorganism *Hafnia alvei* at the meat storage temperature10 °C, and *Carnobacterium*, *Serratia*, and *Yersinia*at 4 °C. The research [53] allowed establishing that ground beef, when stored under aerobic conditions (at 5–7 °C), spoilt after 9 days, and the predominant spoilage microorganisms were *Pseudomonas*, although a number of others were found too: *Enterococcus*, *Kurthia*, *Lactobacillus*, *Leuconostoc*, *Listeria*, *Micrococcus*, *Aeromonas*, *Escherichia*, *Moraxella*, *Morganella*, *Pantoea*, *Providencia*, and *Psychrobacter*.

Holm, Schäfer Koch, and Petersen in their work [54] found that saveloy sausage stored at 5 °C in a modified atmosphere (70% N_2, 30% CO_2) spoilt after 4 weeks, and the main spoilage microorganisms were *Brochothrix thermosphacta*, *Chryseomonas luteola*, *Carnobacterium maltaromaticum*, and *Leuconostoc carnosum*. In 2017, apaper was published [41], which indicated that spoilage of poultry meat stored at 8°C occurred after 4 days, the spoilage microorganisms being*Pseudomonas fluorescens*, *Aeromonas salmonicida*,and *Serratia liquefaciens*. It was also established that *Pseudomonas fluorescens* caused spoilage of poultry meat even at 4°C [40].

Rahnella aquatilis caused spoilage of vacuum-packed pork after 8 days of storage at 2°C [44]. Rabbit meat, which was stored under aerobic conditions at 3°C for 7 days, spoilt when the total number of microbes, psychrotrophs, and *Pseudomonas* reached 8 log CFU/g [47,55].

In the work [48], it was shown that vacuum-packed venison meat stored at 2°C for 14 days spoilt due to the development of *Clostridium gas genes*, which also causes ‘blown pack’ spoilage. And the paper [38] states that the shelf life of vacuum-packed venison at -1 °C is 18 weeks before spoilage.

It is also known that spoilage of packaged pieces of wild boar stored at 0 °C occurs after the 35th day of storage and is accompanied by an unpleasant smell [49]. It was also established that under the same conditions, after 84–98 days, an unacceptable change in colour and an unpleasant smell of *Musculus Longissimus*, which is responsible for the marbling of the meat, were observed.

The work [56] was interesting too: it was found that the total number of microbes in vacuum-packed ostrich meat refrigerated for 2 weeks was below 6 log CFU/g. After 14 days from the date of packaging, the sensory parameters of the meat were as follows. The smell and colour had undergone changes: most of the surface had turned brown, and in general, after 21 days of refrigerated storage, the ostrich meat had become unconsumable. This allowed the scientists to recommend consuming ostrich meat within 10 days from the date of manufacture.

Based on the above, Table 1 lists the genera of bacteria that are often found in fresh meat.

Table 1 – Genera of bacteria most often found in meat and poultry*

Genus	Gram staining	Fresh meat	Poultry meat
Acinetobacter	-	XX	XX
Aeromonas	-	XX	X
Alcaligenes	-	X	X
Arcobacter	-	X	
Bacillus	+	X	X
Brochothrix	+	X	X
Campylobacter	-		XX
Carnobacterium	+	X	
Caseobacter	+	X	
Citrobacter	-	X	X
Clostridium	+	X	X
Corynebacterium	+	X	XX
Enterobacter	-	X	X
Enterococcus	+	XX	X
Erysipelothrix	+	X	X
Escherichia	-	X	
Flavobacterium	-	X	X
Hafnia	-	X	
Kocuria	+	X	X
Kurthia	+	X	
Lactobacillus	+	X	
Lactococcus	+	X	
Leuconostoc	+	X	
Listeria	+	X	XX
Microbacterium	+	X	X
Micrococcus	+	X	XX
Moraxella	-	XX	X
Paenibacillus	+	X	X
Pantoea	-	X	X
Pediococcus	+	X	
Proteus	-	X	X
Pseudomonas	-	XX	XX
Psychrobacter	-	XX	X
Salmonella	-	X	X
Serratia	-	X	X
Shewanella	-	X	
Staphylococcus	+	X	X
Vagococcus	+		XX
Weisella	+	X	
Yersinia	-	X	

*Source: reference[10];+ – gram-positive and bacteria;- – gram-negative bacteria; X – sometimes found; XX – widespread

Contaminants of meat and its processing products are also fungi. They can cause product spoilage by producing mycotoxins, which in turn poison the meat and make it unfit and unsafe to eat. The predominant representatives of fungi isolated from cattle carcasses are *Aspergillus flavus* and *Aspergillus niger* [35]. *Aspergillus* and *Penicillium* were isolated from more than 70% of canned beef and poultry samples [57]. Xerophilic species of *Aspergillus*, *Eurotium*, and *Penicillium* are able to tolerate low pH and high salt concentration, that is why they can grow on the surface of dry-cured meat products [58]. Scientists also found *Penicillium aurantiogriseum* and *Penicillium commune* on dry-smoked products [59].

A characteristic feature of spoilage of meat products under the influence of some fungi is the formation of black, white, or blue-green spots (colonies) on the surface of a product. That made possible to isolate *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Penicillium hirsutum*, and *Aureobasidium pullulans* from spoilt meat with black spots on the surface [60], which allowed other scientists to continue studying the spoilage of meat products with coloured spots formed. Thus, it was found that *Cladosporium oxysporum* formed black spots on dry-cured fermented sausages [61]. Besides, it was described that the black spot spoilage on dry-cured ham was caused by *Cladosporium oxysporum*, *Cladosporium cladosporioides*, and *Cladosporium herbarum* [62]. White spots on frozen meat are a sign of spoilage caused by the growth and development of *Chrysosporium pannorum*, while *Penicillium expansum* is responsible for blue-green spots. *Thamnidium elegans* and *Mucor racemosus* fungi can also cause meat spoilage. The peculiarity of these fungi is that they grow very slowly at low temperatures. The fungus *Cladosporium herbarum* forms visible colonies after 4 months at -5°C [63].

Yeast is another group of foreign microflora that can spoil meat. The peculiarity of yeast is that it can grow in products with a high content of sugar and organic acids or with low water activity and low pH. A characteristic feature of meat spoilage under the action of yeast is the formation of gas and an unpleasant smell [64]. The most common representatives of yeast isolated from fresh meat were *Candida*, *Rhodotorula*, *Debaryomyces*, and *Trichosporon spp.*, while the predominant species in dry-cured and smoked meat products were *Debaryomyces hansenii*, *Yarrowia lipolytica*, *Candida zeylanoides*, *Trichosporon ovoides*, *Trichosporon beigelii*, *Cryptococcus albidus*, and *Rhodotorula mucilaginosa* [65].

For vacuum-packed beef, the most common yeasts were *Candida zeylanoides*, *Kazachstania psychrophila*, and *Candida sake* [66]. Other yeasts known to be present are *Candida alimentaria*, *Candida argentea*, *Cryptococcus carnescens*, *Cryptococcus curvatus*, *Cystofilobasidium macerans*, *Filobasidium uniguttulatum*, *Mrakia frigida*, *Mrakia robertii*, *Pichia fermentans*, and *Rhodotorula mucilaginosa*.

Candida zeylanoides, *Debaryomyces hansenii*, *Candida sake*, and *Candida alimentaria* spoiltmodified atmosphere packed products of meat processing before the end of their shelf life,causinggas formation,blown packs, slime formation, discolouration, and unpleasant smell [67].

The main genera of fungi and yeasts that are often found in meat, including poultry meat, are presented in Table2.

Table 2 – Genera of fungi and yeasts most often found in meat, including poultry meat

Genus	Fresh and chilled meat	Poultry meat
Mould fungi		
Alternaria	X	X
Aspergillus	X	X
Aureobasidium	X	
Cladosporium	XX	X
Eurotium	X	
Fusarium	X	
Geotrichum	XX	X
Monascus	X	
Monila	X	
Mucor	XX	X
Neurospora	X	
Penicilliun	X	X
Rhizopus	XX	X
Sporotrichum	XX	
Thamnidium	XX	
Yeast		
Candida	XX	XX
Cryptococcus	X	X
Debaryomyces	X	XX
Hansenula	X	
Pichia	X	X
Rhodotorula	X	XX
Saccharomyces		X
Torulopsis	XX	X
Trichosporon	X	X
Yarrowia		XX

*X – sometimes found; XX – widespread.Reference source: [10,68-70]

To sum up, scientists have established that all microorganisms, but primarily bacteria, usually reproduce in meat due to the transformation of one or more low-molecular-weight substances in meat, which are utilised by microorganisms in different order, with the production of various by-products depending on the type of microorganism and the availability of oxygen in the atmosphere of the package [9]. The best-known pathogens of meat processing products are *Pseudomonas*, *Acinetobacter*, *Brochothrix*, *Flavobacterium*, *Psychrobacter*, *Moraxella*, *Staphylococcus*, *Micrococcus*, lactic acid bacteria (LAB), and *Enterobacteriaceae*. Important components of meat products, which are substrates for specific spoilage-causing microorganisms, are usually glucose, lactic acid, some amino acids, urea, etc. They are a source for the growth and development of foreign microflora [27].

It was established that glucose transformation products were the precursors of the unpleasant smell formed during meat storage. Other precursors of the unpleasant smell of meat are acetate, acetoin, diacetyl, acetic acid, isobutyric acid, isovaleric acid, 2-methylbutyric acid, 3-methylbutanol, 2-methylpropanol, and ethanol [28].

Table 3 shows the main components of muscle tissue that are used as substrates for reproduction, and some by-products of the metabolism of the most important microorganisms causing meat spoilage.

In a significant part of the reviewed sources, one can often find the results of studying certain microorganisms, which usually cause spoilage of meat products. This deserves detailed consideration.

The genus *Pseudomonas* comprises gram-negative, motile, aerobic, a sporogenous bacilli and also includes five phylogenetic groups based on rRNA similarity analysis [74,75]. The most important species that cause meat spoilage belong to the first group (group I) [76]. This group also includes fluorescent (biovars A-D *P.fluorescens*, biovars A *P.putida* and *P.lundensis*) and non-fluorescent (biovars 1 and 2 *P. fragi*) species. Due to their rapid reproduction under aerobic conditions, *Pseudomonas* can cause spoilage of raw meat. If the amount of *Pseudomonas* reaches 10^7–10^8 CFU/g, mucus and an unpleasant smell are found on the surface of the meat. This is explained by the fact that after the main substrates (glucose and lactate) run out, the bacteria begin to convert amino acids [27]. Besides, *Pseudomonas* are the predominant microorganisms that cause spoilage of chill-stored meat under aerobic conditions [74].

Shewanella putrefaciens is an aerobic, gram-negative, rod-shaped bacterium. Its taxonomic status is still uncertain [76]. Analysis of animal products showed that many of them contained this bacterium. *Shewanella putrefaciens* is able to produce H_2S, which binds to myoglobin, turns the meat green, contributes to the unpleasant smell of rotten eggs, and thus makes meatinedible. Besides, this bacterium can spoil chilled meat even in vacuum packaging [77].

Lactic acid bacteria (LAB) are a group of microaerophilic microorganisms that predominate in vacuum-packed or MAP fresh meat of cattle and poultry. These microorganisms include *Lactobacillus* (mainly *Lact. sakei/curvatus* [78]), *Leuconostoc*, *Carnobacterium* [79-85]. Also, in addition to those listed, lactic acid bacteria that cause spoilage include *Weisella* (mostly *W. Viridescens*, *W. Paramesenteroides* [87]), *Lactococcus* (*Lc. raffinolactis*), *Enterococcus*, and *Pediococcus* [32,82,83,87]. LAB are divided into homo- and heterotrophic types [87]. Homofermentative LAB produce only lactic acid, which tastes sour, whereas heterofermentative LAB produce compounds that are associated with meat spoilage, including ethanol, sulphides, lactate, acetate, and, most importantly, butyric acid with its characteristic rancid taste. This spoilage occurs when the number of microbes reaches 10^8 CFU/cm^2 [87]. *Lactobacillus sakei* produces H_2S, which turns myoglobin into green-coloured sulphomyoglobin (this type of spoilage is characterised by a limited amount of glucose and oxygen) [32].

Psychrotrophic representatives of the sporogenous genus *Clostridium* are often associated with spoilage of vacuum-packed fresh meat (‘blown pack’) [88-90].

Table 3 – The main components of muscle tissue that are used as substrates for reproduction, and some by-products of the metabolism of the most important microorganisms causing meat spoilage

Microorganism	Substrate[a]		The main end products of metabolism	
	Aerobic	Anaerobic	Aerobic	Anaerobic
Pseudomonas	Glucose [1] Amino acids [3] Lactic acid [2]	-	MucusSulphides, ethers, acids(gluconate), amines	
Acinetobacter/Moraxella	Amino acids[1] Lactic acid[2]	-	Ethers, nitrites, oximes, sulphides	-
Shwanella putrefaciens	Glucose[1] Amino acids[1,2] Lactic acid[3]	Glucose[1] Amino acids[1]	Volatile sulphides	H_2S
Brochothrix thermosphacta	Glucose [1] Amino acids [2] (glutamate)	Glucose[1]	Acetic acidAcetoinIsovaleric acidIsobutyric acid	L-lactic acid Volatile fatty acids
Enterobacter	Glucose[1] Glucose-6-phosphate[2] Amino acids[3] Lactic acid[4]	Glucose[1] Glucose-6-phosphate[2] Amino acids [3]	Sulphides Amines	Lactic acid CO_2, H_2 H_2S Amines
Lactobacillus	-	Glucose[1] Amino acids[2]	-	D, L-lactic acid Acetic acid Volatile fatty acids

[a]Superscript numbers indicate the order of disposal of the substrate.

Literature sources: [9,18,71-73]

One of the most famous representatives of this genus is *Clostridium esterthеticum*, which causes so-called blown pack spoilage. As a rule, meat that can be affected by *Clostridium estertheticum* undergoes organoleptic changes when spoilt (its colour changes, its consistency becomes soft, and a strong unpleasant smell appears) [91]. Also, clostridia can grow and reproduce in vacuum packaging, while fermenting glucose with the formation of butyric acid, butanol, CO_2, and H_2, which also lead to meat discolouration [88]. *Clostridia* are the cause of spoilage of chilled vacuum-packed beef, which is characterised by specific odours (sulphurous, fruity, solvent-like, and even “strong cheese”) [88]. It is known that unpleasant cheesy and milk smells in vacuum-packed chilled lamb meat are also characteristic of the development of *Clostridium spp.* [51].

Brochothrix thermosphacta is known as a spoilage agent, a gram-positive bacterium capable of enzymatic metabolism and producing L-lactate from glucose [9]. It is most often found during spoilage of vacuum-packed chilled meat. The best substrate for its development in meat is glucose. Under aerobic conditions, it can produce acetoin, acetic acid, isobutyric acid, 2-methylbutyric acid, isovaleric acid, and 3-methylbutanol. It is also characterised by the appearance of a specific smell – cheesy, associated with the formation of acetoin/diacetyl and 3-methylbutanol [30,92].

The best-known meat-spoiling members of the *Enterobacteriaceae* familyare*Serratia*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Proteus*, and *Hafnia*, namely*Serratia liquefaciens*, *Hafnia alvei*, and *Enterobacter (Pantoea) agglomerans* [31]. These bacteria convert first glucose, and then amino acids, producing amines, sulphides, and H_2S [28].

Of all microorganisms that contaminate raw meat, yeast and moulds are the least involved in its spoilage. Also, they have fewer opportunities to reproduce in it than bacteria do.

Deterioration of the sensory characteristics of meat products is also associated with moulds that produce lipases, proteases, and carbohydrases. Moulds are known to produce volatile organic substances, such as dimethyl disulphide, geosmin, and 2-methylisoborneol, which negatively affect a product’s quality [93].

Yeast, as well as moulds, is also known for producing various bioproducts of vital activity that lead to deterioration of the sensory properties of the product. Yeast needs oxygen for optimal reproduction. It is known that their competitiveness and ability to spoil meat products decrease in case of vacuum or MAP storage, and accordingly increase in case of oxygen-accessed packaging and storage in a refrigerator.

Factors affecting the growth and development of foreign microflora in meat and its processing products are quite diverse, which makes it difficult to solve the problem of extending the shelf life of meat food products, as it requires the selection of a wide range of parameters. On the other hand, managing these factors makes it possible, by selecting their combination, to extend the shelf life of meat products.

The growth and development of meat-spoiling microorganisms depends on a number of factors, which can generally be divided into two groups: external and internal. Internal factors include physical and chemical properties, such as the type and age of slaughter animals, initial microflora, nutrient content, water activity, and pH. External factors include product storage conditions, such as the temperature and composition of the packaging atmosphere, other factors, such as the primary processing of the carcass and methods of technological processing of raw materials, etc. [94]. Let us consider the main factors in detail.

The internal factors that most often directly determine the shelf life of food by affecting the growth and development of microorganisms include pH, water activity, and the presence of preservatives.

The pH of meat is one of the key internal factors. It affects the survival, growth, and development of microorganisms, because each species has its own optimal pH and metabolic activity. It is known that the slaughter of animals stops the enzymatic activity in the body, which in turn inhibits glycolysis, therefore, for normal post-slaughter meat, the pH is in the range 5.4–5.8. At the same time, in hard and dry meat obtained from animals subjected to stress before slaughter and in cooked meat products such as sliced ham, the pH was found to be>6 [95]. It was established that a high pH and the presence of adipose tissue accelerate the growth of bacteria and, consequently, the spoilage of a product [96].

The growth of *B. thermosphacta* is characterised by low chilling temperatures (about 4°C) and a high pH [46]. On the other hand, *Enterobacteriaceae* are unable to grow under anaerobic conditions in beef if its pH value is below 5.8 [50]. Due to the work [56], it was established that the number of bacteria in chilled vacuum-packed ostrich meat increased in proportion to the pH value in the meat.

It is definite that LAB produce lactic acid, which slightly lowers the pH, though in their absence, the pH of the meat does not change much. It is also known that most pseudomonads produce the enzyme metallopolteinase, which requires a pH between 6.5 and 8.0 for its growth [41].

The activity of water (a_w) and the presence of preservatives, in particular, table salt, are also determining internal factors affecting the vital activity of microorganisms in meat and its processing products.

Water activity shows the degree of saturation with water that is present in the product and is available for growth, that is, for enzymatic reactions and all biochemical processes in the cells of microorganisms. This indicator in meat and meat products represents the relative humidity of the air in equilibrium with the product [98]. According to the content of active moisture, food products can be classified into the following categories:

• wet products that have an a_w> 0.85 and require chilling or other methods to regulate the growth of pathogenic(i.e. foreign) microflora;

• semiwet products, with the a_w 0.60–0.85 and a limited shelf life, are more susceptible to damage by yeast and mould fungi;

• products with a low moisture content, which have an a_w< 0.60, with an extended shelf life, are stored normally.

Water activity in fresh meat is a_w> 0.85, which makes it possible to state that meat is a perishable product. Some microorganisms can actively develop at a certain minimum a_w value, which has been established for each of them. As a rule, the best growth of microorganisms is observed at the a_w values 0.98–0.99, and at a_w< 0.90, they stop growing. But yeast and mould fungi can grow at a far lower value of a_w<0.60. Table 3 shows the optimal values of water activity for the growth of some microorganisms [10,99-100].

Table 4 – Optimal values of water activity for some microorganisms

Microorganisms	a_w
Bacteria	
Campylobacter	0.98
Pseudomonas	0.97
Clostridium botulinum type E	0.97
Pseudomonas fluorescens	0.97
Bacillus cereus	0.95
Escherichia coli	0.95
Clostridium botulinum type A	0.95
Salmonella spp	0.95
Staphylococcus aureus	0.86
Mycelial fungi	
Mucor plumbeus	0.93
Alternaria citri	0.84
Penicillium chrysogenum	0.79
Aspergillus flavus	0.78
Aspergillus Niger	0.77
Yeast	
Saccharomyces cerevisiae	0.90

So, it can be stated that this indicator is one of the determinants for the growth and reproduction of any microflora. Therefore, by changing its numerical value, it is possible to control the vital activity of spoilage microorganisms and thus extend the shelf life of a product.

For example, the best way of processing meat to reduce water activity is drying. The shelf life of a product can also be extended by chilling or adding preservatives (table salt and sugar), etc. Sodium chloride (salt) is one of the most popular preservatives used in the meat industry due to its low cost, preservative and antimicrobial properties, etc. Salt can reduce the water activity of meat and of its processing products. Adding salt to meat products is known to affect some enzymes and thus improve the moisture retention and taste of the meat [101].

The values of water activity were established for some products of meat processing and given in the literature [99,100].

External factors, as well as internal, are also effective, as they are usually a physical obstacle to the settlement of foreign microflora on meat products.

Both the meat storage conditions and the composition of the atmosphere of the packaging affect the spoilage of meat and its processing products [102,103]. The main spoilage bacteria in aerobic storage are *Pseudomonas spp.*, *Acinetobacter spp.*, and *Moraxella spp*. For meat packed in the presence of air, the main spoilage bacteria are *P.fluorescens*, psychrotrophic *P. fragi*, *P. lundensis* and *P. putida* [104]. For meat packaging kept under vacuum and high pH values in vacuum, the typical spoilage-causing genus is *Shewanella*, namely the dominant species *S.putrefaciensis* [31]. An atmosphere with a high concentration of carbon dioxide (CO_2) or vacuum packaging significantly extends the shelf life of meat and its processing products, as compared with conventional packaging methods [105].

Nitrogen (N_2) and CO_2 are widely used for food packaging. However, they prolong the lag phase of aerobic microorganisms and, too, stimulate the growth of facultative and obligate anaerobes. LAB are the dominant spoilage bacteria for vacuum-packed or CO_2-modified-atmosphere-packed meat products. The growth of gram-negative spoilage bacteria, as well as the increase in LAB, is facilitated by microaerophilic conditions with reduced water activity [106,107].

There are a number of factors determining the effectiveness of modified atmosphere packaging (MAP) of meat:

• changes in the composition of the free space, i. e. the concentration of CO_2 gases during storage;

• temperature;

• Packaging configuration;

• meat characteristics.

The most popular bacteria isolated from MAP meat is *Serratia spp.*, which belongs to the *Enterobacteriaceae* family [31].

Temperature is the main physical factor that affects the duration of storage of meat and its processing products due to changes in the lag phase, specific growth rate, and number of microbial cells. Different microorganisms have their own optimal temperature at which they begin growing actively. For example, for psychrophilic bacteria, the temperature range is -5 to 20°C, and the optimal temperature for their growth and development ranges 5 to 15°C. For mesophilic microorganisms, the optimal temperature is 20–25°C, for thermophiles, it ranges 45 to 60°C, and for extreme thermophiles, this range is 85 to 90°C. It is well known that a lower storage temperature reduces the growth of bacteria, including that of spoilage bacteria.

During vacuum storage at 1.5°C, the predominant spoilage microorganism is *Carnobacterium spp.*, while at 4°C and 7°C, it is homofermentative *Lactobacillus spp*. From the *Enterobacteriaceae* family, *Serratia liquefaciens* and *Hafnia alvei* are the main dominant bacteria prevailing at the storage temperatures 1.5°C and 4°C respectively.

Carnobacterium, *Yersinia*, *and Clostridium spp.* were the predominant microorganisms causing spoilage of vacuum-packed lamb stored at -1.2°C, while at 8°C, the main spoilage bacteria were *Hafnia*, *Lactococcus*, and *Providencia spp.* [52].

The paper [63] says that moulds causing meat spoilage developed at -5°C, although low temperatures slowed down their development, and visible colonies began appearing only after 4 months.

In [66], it is reported that after inoculation of beef with *K. psychrophila* yeast in the amount 2 log CFU/cm^2 and vacuum packaging, the amount of yeast increased to 5 log CFU/cm^2 after only 16 days at the storage temperature 4°C.

Results of the research and their discussion

Since this paper is a review and generalisation, its data will help to systematise the information accumulated by other leading specialists from around the world, related to the spoilage of various meat types and the effect of various external and internal factors. The object of our further research will be raw poultry meat, like chicken, so in the discussion, we want to focus on this very type of meat.

Currently, the most well-known and widespread bacteria that cause spoilage of raw meat are*Acinetobacter/Moraxella*, *Psychrobacter*, *Pseudomonas*, *Aeromonas*, *Shewanella putrefaciens*, *Brochothrix thermosphacta*, *Flavobacterium*, *Staphylococcus*, *Micrococcus*, *Enterobacteriaceae*, lactic acid bacteria of the genera *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, *Weisella* [13,31].

The first place where raw meat can be contaminated are specialised buildings (abattoirs, or slaughterhouses), and the bacteria released there are *Enterobacter*, *Citrobacter*, and *Klebsiella*. In addition, some cases of contamination by foreign microflora are due to slaughterhouse workers’ non-compliance with sanitary requirements, which is another source of contamination. Also, critical part of contamination can be detected during the removal of the animal’s entrails and when gutting the bird, because the intestines and feathers are the main source of hazard at this stage [34].

The most common way of storing chicken is chilled storage, when the specific spoilage bacteria are *Pseudomonas*, *Enterobacteriaceae*, lactic acid bacteria, and *Brochothrix thermosphacta* [40,41].

Chicken stored in modified atmosphere package also spoils, but this spoilage occurs after 6–7 days at the storage temperature 10°C and after 9–10 days at 4°C [53]. The scientists isolated the microorganisms *Brochothrix thermosphacta*, *Carnobacterium*, and *Pseudomonas*, which caused the spoilage of meat stored at 4 and 10 °C and packed in a modified atmosphere (80% O_2 and 20% CO_2). From modified atmosphere packed (65% N_2 and 35% CO_2) chicken, the specific spoilage microorganisms isolated were: *Hafnia alvei* at the meat storage temperature 10 °C and *Carnobacterium, Serratia*, and *Yersinia*at 4°C.

Pseudomonas fluorescens, *Aeromonas salmonicida*, and *Serratia liquefaciens* were identified after 4 days of storage at 8°C [41], while at the storage temperature 4 °C, only *Pseudomonas fluorescens* was detected [40].

Our analysis of the latest research and publications has allowed us to present in Table 1 (taken from [10]) the most complete list of genera of bacteria most often found in fresh meat. According to these data, there are genera of bacteria that typically develop in the thickness of both red meat (beef, pork, game meat, etc.) and poultry meat (chicken, ostrich meat, etc.). Thus, the most common genera of bacteria characteristic of all meat types are *Acinetobacter* and *Pseudomonas*. For red meat, the commonest genera are *Aeromonas*, *Enterobacter*, *Moraxella*, and *Psychrobacter*. And for poultry, the most common bacterial genera are *Corynebacterium*, *Listeria*, *Micrococcus*, and *Vagococcus*.

The presence of such genera of bacteria as *Bacillus*, *Brochothrix*, *Citrobacter*, *Clostridium*, *Enterobacter*, *Erysipelothrix*, *Flavobacterium*, *Kocuria*, *Microbacterium*, *Paenibacillus*, *Pantoea*, *Proteus*, *Salmonella*, *Serratia*, and *Staphylococcus* is also characteristic of red meat and poultry meat.

It has been established that each type of meat has its own, specific genera of bacteria that can cause spoilage of the food product. For example, for red meat, the specific bacteria are *Escherichia*, *Hafnia*, *Kurthia*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Shewanella*, *Weisella*, and *Yersiinia*, and for poultry, *Campylobacter* and *Vagococcus*.

Besides bacteria, meat spoilage can also be caused by other microorganisms –moulds and yeasts. Their main sources are air, wastewater, working surfaces and walls of the slaughterhouse, equipment and workers of the enterprise [35,36]. Of course, as it is with bacteria, non-observance of sanitary and temperature conditions when transporting carcasses to the main production site can result in contamination with these foreign microorganisms, too [39]. Yeast, the specific feature of which is its ability to grow in an environment with a high amount of sugar and organic acids or with low water activity and low pH, is somewhat less common than mould fungi are. A visible sign of contamination of a meat product with yeast is the presence of gas and an unpleasant smell [64].

Aspergillus flavus and *Aspergillus niger* are the most common representatives of fungi detected in cattle carcasses [35]. And in canned beef and poultry meat, not only were representatives of the genus *Aspergillus* found, but of *Penicillium* too [57]. The most common genera of yeast isolated from fresh meat were *Candida*, *Rhodotorula*, *Debaryomyces*, and *Trichosporon spp.* [65].

Among moulds and yeasts, the same tendency is observed as among bacteria. Red meat and poultry are characterised by common genera of spoilage fungi: *Alternaria*, *Aspergillus*, *Cladosporium*, *Geotrichum*, *Mucor*, *Penicillium*, and *Rhizopus*. The most common genus of yeast present both in red and in poultry meat, is *Candida*.

Besides, it has been established that moulds more characteristic of red meat are *Cladosporium*, *Geotrichum*, *Mucor*, *Rhizopus*, *Sporotrichum*,and *Thamnidium*, and specific fungi only present in red meat are *Aureobasidium*, *Eurotium*, *Fusarium*, *Monascus*, *Monila*, and *Neurospora*.

The most common yeast present in red meat is *Torulopsis*, while in poultry, the commonest ones are *Debaryomyces*, *Rhodotorula*, and *Yarrowia*. The common yeast species found in both poultry and red meat are *Cryptococcus*, *Debaryomyces*, *Pichia*, *Rhodotorula*, *Torulopsis*, and *Trichosporon*. The yeast genus specific to red meat is *Hansenula*, and the ones specific to poultry, are *Saccharomyces* and *Yarrowia*.

To sum up, we would like to point out certain regularities:

– out of the forty genera found in all meat types, 27 genera (67.5%) are characteristic of poultry meat. This indicates a poorer generic diversity of bacteria, which to some extent is an advantage of this type of meat.

– poultry meat has been found to have a poorer generic diversity of mould fungi too (7 genera out of the 15 registered ones), which also indicates the advantages of poultry meat.

– this distribution is not observed for yeasts, since most genera (9 out of 10) are found both in chicken and in other types of meat.

Verifying the research results

Active water, as a part of total water that is not connected to biopolymers, with its numerical value ranging 0.00 to 1.00, is to some extent a marker indicator that allows determining the shelf life of a food product. The smaller this indicator is, the longer the shelf life of meat and products of its processing [108,109], since microorganisms grow and develop when the numerical indicator of water activity is within the range 0.65 to 1.00. Therefore, the lower the value of this indicator, the longer the product will be suitable for consumption, because the survival of microorganisms will decrease. Of course, this indicator can also be used to evaluate the vital activity of bacteria found in meat and its processing products, their resistance to heat treatment, and susceptibility to spoilage [110]. The values of the indicator a_w that have been established to date for a number of food microorganisms are listed in Table 1 [10]. This indicator characteristic of a certain type of microorganisms can be correlated, as it depends on a number of other factors too: the initial microbial contamination of the product, pH, temperature, storage conditions, etc., and constantly changes during the storage of meat products.

In the meat industry, water activity is a parameter traditionally used to evaluate meat processing products. As a rule, it is in the range 0.80 to 1.00 and is presented in several works [99,100].

According to the indicator of water activity established for a number of microorganisms, they fall into several groups: sensitive (minimum values of a_w = 0.95–0.99); low-sensitive (minimum values of a_w = 0.93–0.95); resistant (minimum values of a_w = 0.90–0.93); highly resistant (minimum values of a_w< 0.90) [99,100].

Thus, water activity is an important physical parameter in studying the quality and safety of meat, as it is one of the determining factors in the growth, development, and reproduction of microorganisms. Consequently, this parameter, in our opinion, is one of the key factors affecting the stability of food products during storage. This physical criterion can be adjusted in meat products, which will allow extending their shelf life. In the future, we would find it interesting to investigate how water activity correlates with other characteristics, in particular, with microbiological indicators characterising the deterioration of meat during its storage. This will allow improving the existing technologies of storing meat products, and may be the basis for the development and implementation of new ones.

Conclusion

In today's world, the demand for high-quality and safe food products is constantly growing. But the manufacture of product is always accompanied by losses: economic, product, technological, etc. The main goal in the new world is to use rationally all available resources, and this can only be achieved if one knows the technology, the specific features of the industry, and the factors affecting the way of processing the product.

All of this also applies to the meat industry, which is constantly improving due to new discoveries in how to identify spoilage microorganisms, new types of packaging, new methods of product processing, and new preservation methods. In the meat industry, a key point that helps reduce the amount of spoilt products is the effect of external and internal factors on the meat and their interaction with each other.

This work has considered the basics of food spoilage, described briefly the main microorganisms that cause the spoilage of raw meat, and analysed their diversity. And, most importantly, it has considered and described the internal and external factors affecting the rate and intensity of spoilage of raw meat, and substantiated their relationship with the growth of spoilage microorganisms.

All the issues considered above create favourable conditions for the development of new ways to extend the shelf life of meat products, detect spoilt products at early stages, and prevent possible risks of contamination of products with pathogenic microflora. This will help reduce resource losses at all stages of the manufacture of meat products.

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ЗНАННЯ ПРО ФАКТОРИ, ЩО ВПЛИВАЮТЬ НА ТРИВАЛІСТЬ ЗБЕРІГАННЯ СИРОГО М'ЯСА – ЗАПОРУКА РАЦІОНАЛЬНОГО ВИКОРИСТАННЯ ВИРОБНИЧИХ РЕСУРСІВ

А.О. Логінова,аспірантка, *E-mail*: anna.ol.logi@gmail.com
Л.Ю. Арсеньєва, доктор технічних наук, *E-mail*:ars-l@ukr.net
Кафедра експертизи харчових продуктів
Національний університет харчових технологій,вул. Володимирська, 68, м. Київ, 01033

Анотація. У даній роботі, що має оглядовий та узагальнюючий характер, наведено та систематизовано накопичену іншими провідними фахівцями всього світу інформацію, що стосується зберігання та особливо псування різних видів м'яса. Розглянуто основні фізичні фактори, що викликають псування харчових продуктів м'ясного походження, джерела забруднення на всіх етапах ланцюга виробництва м'ясних продуктів, починаючи з надходження сировини на виробництво і до етапу пакування у різну споживчу тару та зберігання за різних температурних умов. Коротко описано основні представники сторонньої мікрофлори (бактерії, плісняві гриби та дріжджі тощо), що викликають псування сирого м'яса; проаналізовано їх таксономічну різноманітність та з'ясовано характерні діагностичні ознаки псування м'яса мікроорганізмами певних груп. Крім того, розглянуто та описано внутрішні та зовнішні фактори, що є визначальними для росту, розвитку та розмноження мікрофлори, а особливо тієї, що впливає на швидкість та інтенсивність псування сирого м'яса, обґрунтовано їх взаємозв'язок і взаємовплив. Встановлено певні закономірності впливу цих факторів на життєдіяльність мікроорганізмів псування,змінюючи числове значення яких, можна впливати на їх ріст, розвиток та розмноження і, таким чином, подовжувати строки придатності продукту. Наприклад, відомо, що один з найрозповсюдженіших фізико-хімічних показників м'яса, що впливає на його спосіб перероблення чи термін придатності є показник активної кислотності. Відомий факт, що при збільшенні даного показника зменшується термін зберігання м'ясної продукції. У той же час, такий показник як активність води є важливим фізичним параметром при дослідженні якості та безпечності м'яса, оскільки є одним з вирішальних для росту і розвитку мікроорганізмів. Відповідно, цей параметр, на нашу думку, є одним з ключових, що впливають на стійкість та тривалістьм'ясних продуктів під час зберігання і тому корегування його числового значення різними способами дасть можливість удосконалити вже існуючі технології зберігання м'ясних продуктів, а можливо і розробку та впровадження нових.

Ключові слова: мікробіологічне псування м'яса, стороння мікрофлора, активність води, швидкопсувний продукт, бактерії, плісняві гриби, дріжджі, курятина.