

VETERINARY AND SANITARY ASSESSMENT AND DISINFECTION OF  
REFRIGERATOR CHAMBERS OF MEAT PROCESSING ENTERPRISES*Kateryna Rodionova, Anatoliy Paliy, Mariia Khimych***ABSTRACT**

The results of microbiological studies of air samples of refrigerating chambers of meat processing enterprises are presented. The quantitative composition of the air microbiota of the chambers of the refrigerating shop was studied. It has been established that the technological regimes for cooling meat in cooled chambers ( $t = +4\text{ }^{\circ}\text{C}$ ) and deep freezing chambers ( $t = -18\text{ }^{\circ}\text{C}$  and  $-22\text{ }^{\circ}\text{C}$ ) have no bacteriostatic effect on the life activity of mold fungi. The developed disinfecting preparation (hydrogen peroxide (8.0 – 10%), acetic acid (10%), peracetic acid (5.0 – 7.0%), stabilizing additives, water) ensures the destruction of sanitary-indicative microorganisms in cold rooms meat processing plants when applied at a concentration of 0.05% – 60 minutes, 0.1% – 30 minutes, 0.15% – 10 minutes and does not have a toxic effect on meat raw materials that are stored in chambers of the refrigeration shop after disinfection.

**Keywords:** refrigerating chambers; microorganisms; disinfectant; concentration; exposure

**INTRODUCTION**

Today, refrigeration is the most common and effective method of preserving meat products. Ensuring the quality and safety of raw meat during long-term storage at low temperatures primarily depends on the creation of a continuous refrigeration chain throughout the production of the product – from production to consumer (Ryabchenko, 2016; Göransson, Jevingerb and Nilssonc, 2018; Ishevskiy and Davydov, 2017).

During refrigerated storage, the properties of products change due to the processes that occur in them (physical, biochemical, microbiological, etc.). In some cases, they improve the consumer properties of products, and in others – cause their spoilage (Bruckner et al., 2012; Stonehouse and Evans, 2015; Bermúdez-Aguirre and Welti-Chanes, 2016). Therefore, the purpose of refrigeration and storage of products is to ensure the favorable passage of the first (meat autolysis, etc.) and minimize other (drying, microbiological changes, oxidation, etc.) processes (Kaalea et al., 2011).

It should be noted that maintaining the proper quality of products during their long-term storage also depends on the general sanitary and hygienic conditions of refrigerators. Without timely washing and disinfection, pathogenic bacteria actively multiply in refrigerators, mold develops, and as a result, an unpleasant odor appears. This directly affects the quality of meat products, which begin to spoil and become completely unfit for consumption and dangerous to human health (Bogatko and Sakhniuk, 2013).

Low temperature does not destroy microorganisms or enzymes, but only inhibits their activity. According to

temperature resistance, microorganisms are divided into three groups: thermophiles (develop at a temperature of  $20 - 80\text{ }^{\circ}\text{C}$ , optimally at  $50 - 75\text{ }^{\circ}\text{C}$ ), mesophiles ( $5 - 57\text{ }^{\circ}\text{C}$ ), and psychrophiles ( $-10 - 10\text{ }^{\circ}\text{C}$ ). Psychrophiles are the most interesting for refrigeration technology. They are divided into facultative, which can live in the same conditions as mesophiles, and obligate, which can reproduce only at low temperatures. Psychrophilic bacteria actively multiply on products with low acidity – meat, fish, non-acidic dairy products at temperatures of  $-5 - 8\text{ }^{\circ}\text{C}$ . Most molds are psychrophilic and actively develop on frozen foods. They stop their reproduction at  $-2 - 3\text{ }^{\circ}\text{C}$  (certain types of molds – only at  $-8 - 10\text{ }^{\circ}\text{C}$ ). Separate colonies of molds appear on the surface of frozen meat stored at a temperature higher than  $-8\text{ }^{\circ}\text{C}$  (Berk, 2018).

The mycelium of the fungus penetrates the thickness of the product, forming spores (for fungi and yeast, they are not only a means of protection against adverse external factors but also a means of reproduction). Mold begins to multiply, forming white, grey, and black spots on the surface of the product, as a result of which the products of mold activity accumulate in meat products, an unpleasant odor appears (Mohapatra et al., 2017).

Ways of contamination of raw materials, semi-finished products, and finished products by microorganisms are extremely diverse (Dukhnytskyi et al., 2011; Dudchik et al., 2013; Paliy, Rodionova and Paliy, 2017). However, the most important source of additional microbial contamination of meat and meat products during refrigerated storage is the air of the refrigerator. Microorganisms, regardless of the pathogenicity that

accumulates in the air, on the walls and technological equipment, and their products (especially microscopic fungi) in case of contact with raw meat can pose a danger to human health due to infection or food poisoning, so sanitary-microbiological control of air in the chambers of the refrigeration shop has an important veterinary-sanitary significance (**Bogatko and Sakhniuk, 2013; Prokopenko, 2013**).

In the food industry, in production shops, and in places of storage of products the level of microbial air pollution depends on a way of cleaning of rooms, the organization of production process, application and efficiency of work of ventilation and other conditions (**Iliashenko et al., 2008**).

Today, despite the large arsenal of modern disinfectants, the search for new effective drugs is continuing, both through the synthesis of new chemical compounds and through the creation of compositions from existing substances. In composite means, it is possible to supplement the functional properties of each of the components, as well as to achieve the mutual strengthening of the activity of components (synergism) (**Glazova, 2007; Paliy and Rodionova, 2017**).

Analysis of the nomenclature and active substances of the market of veterinary disinfectants registered in Ukraine shows that the main active ingredients of disinfectants are Quaternary Ammonium Compounds (QAC), aldehydes, oxygen-containing compounds, organochlorines, guanidines, and their combinations. The share of means of domestic production is only 45.5% (**Dymko, Pushkova and Solomon, 2015**). In addition, it should be noted that about 50% of the modes of use of disinfectants specified by the manufacturer are not able to provide effective disinfection of facilities. In the instructions for use of these tools, the concentrations of working solutions and exposures are artificially underestimated in the tens or even hundreds of times from those that can cause the death of microorganisms (**Kanishchev and Ereemeeva, 2016**).

Oxygen compounds have become widely used in world veterinary practice. They show a wide range of bactericidal activity, can dissolve blood and many other biological substrates, coagulate protein well, have no odor, rapidly decompose in the environment into non-toxic products (**Paliy, 2018; Rodionova and Paliy, 2017**). Drugs of this group are strong oxidants, the main action of which is the formation of free radicals that disrupt lipid metabolism in the cell membrane, DNA, and other important components of the microbial cell. Despite the reproduction by many microorganisms of catalase, which protects the cell from the effects of peroxides by decomposing them into water and oxygen, the concentrations used during disinfection allow, in most cases, to overcome this mechanism of resistance. The main disadvantages of the drugs of this group are high tissue toxicity (class 2) with local irritant and resorptive effects. At the same time, they can cause corrosion of metals and discolor tissues. It is noted that from the group of oxidants promising is the use of drugs containing peracetic acid and hydrogen peroxide (**Kovalenko and Nedosekov, 2011; Zavgorodnyi et al., 2013; Rodionova, 2017**).

Promising are also compositions that are based on hydrogen peroxide with the addition of organic acid. The peracids, which are formed in this composition, significantly increase the bactericidal action of the disinfectant. Drugs 'Nu-Cidex' (England), 'Clinezin-oxy'

(USA), 'Anioxide-1000' (France) were obtained based on hydrogen peroxide and peracetic acid. Drugs 'Vofasteril' and 'Persteril' are used in medical practice for disinfection abroad (**Kuchma, 2004**).

Along with this, it should be noted that today the range of oxygen disinfectants for use in food technology is quite limited, which led to the relevance of our work on the development of modern import-substituting antimicrobials.

### Scientific hypothesis

Compliance with sanitary and hygienic requirements in meat processing plants during the production of food with good manufacturing practices (GMP), good hygiene practices (GHP), and the implementation of the HACCP system is the key to the production of safe and competitive meat products in each relevant enterprise. The study of bacterial contamination of the air and the sanitary condition of refrigerators is an urgent task of practical veterinary medicine because the improper organization of veterinary and sanitary control causes the risk of contamination of meat with environmental microflora.

We investigate several hypotheses in our study:

1. The low temperature in the deep-freeze chambers does not destroy microorganisms, but only inhibits their activity.
2. Developed disinfectant (hydrogen peroxide (8.0 – 10%), acetic acid (10%), peracetic acid (5.0 – 7.0%), stabilizing additives, water) provides destruction of sanitary- indicative microorganisms and colonies of molds in refrigerators meat processing plants.

## MATERIAL AND METHODOLOGY

### Samples

The experimental part of the work was carried out based on meat processing enterprises of Kharkiv and Volyn oblasts of Ukraine, the laboratory of veterinary sanitation and parasitology of the National Research Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv), and the Department of Veterinary Hygiene, Sanitation, and Expertise of Odesa State Agrarian University, Odesa). During the sanitary and hygienic study of the air, the samples were taken at five different points of the room, not lower than 0.5 m above the floor level by the sedimentation method (**Golovko and Rublenko, 2010**).

### Chemicals

Before placing the raw meat in the cooling and storage chamber, it was thoroughly washed and disinfected using 'Anti-Jerm SR S 25' detergent (Vetsintez LLC, Ukraine) (2.0% – 30 min) and disinfectant 'CD-256' (IPAX Cleanogel, Inc., USA) (0.3% – 1 hour).

To design the formulation of a new disinfectant, we identified the chemical components and selected their optimal ratio. Hydrogen peroxide, acetic, and peracetic acid were used as active substances.

Acetic acid — organic acid, monobasic carboxylic acid, chemical formula  $\text{CH}_3\text{COOH}$ . Danger class - 3, moderately toxic substances (**GOST 12.1.007-76, 1977**).

Peracetic acid — organic peracid (peroxyacetic acid), chemical formula is  $\text{CH}_3\text{CO}_3\text{H}$ . According to the parameters of acute toxicity belongs to the 3<sup>rd</sup> class of danger (**GOST 12.1.007-76, 1977**) when brining into the stomach, to the 4th class of danger when it is applied on the

skin, to the 2nd class of danger according to the classification of inhalation danger. Hydrogen peroxide (Hydrogen peroxide H<sub>2</sub>O<sub>2</sub>, Latin Hydrogenii peroxydum) belongs to peroxy compounds. Danger class – 3 (**GOST 12.1.007-76, 1977**).

#### Animals and Biological Material:

To determine the bactericidal properties of the developed disinfectant, use test cultures of microorganisms stored in the collection of NSC 'IECVM': *Escherichia coli* (strain K 99), *Salmonella Dublin* (strain 41), *Staphylococcus aureus* (strain 209).

The cultures were incubated at a temperature of 37.5 ±0.5 °C on the meat peptone agar (MPA) and meat peptone broth (MPB) for 24 hours.

#### Instruments

The air of the chamber was rehabilitated using a portable vertical irradiator-recirculator of closed type ORUB-01- 'KRONT' (CJSC 'KRONT-M', Russia) for 30 min to achieve air sterility, which was confirmed by microbiological studies.

Before preventive disinfection, the walls, ceiling, and floor of the refrigeration chambers were mechanically cleaned, washed, and degreased with detergents using a KÄRCHER machine.

In front of the entrance to the cell, a sanitary pass SPD 08.04 of the checkpoint type (INOX TIME, Ukraine) was placed to exclude secondary contamination of raw meat during contact with the working staff. Only members of the control and expert group approved by the Order of the enterprise had access to raw meat.

Lamps TUV-30W (Philips, Netherlands) are the source of ultraviolet radiation in laminar boxes ozone-free.

Colonies grown both on the surface and in the depth of the MPB were counted using a colony meter 'SYNBIOSIS aCOLyte' (Synbiosis, USA).

The optical microscope Axioskop 40 (Zeiss, Germany) was used for smear microscopy.

To determine lead, cadmium, copper, zinc, mercury the Atomic Adsorption spectrometer 'Agilent AA 240 FS' with a graphite-thermal add-in-device for atomization 'GTA 120' (Agilent, USA) has been used. Arsenic was determined using a spectrophotometer ULAB 102 UV (ULAB, Ukraine).

Pesticides were determined with a gas chromatograph 'Agilent 7890 A GS System' (Agilent, USA). Organochlorine pesticides were determined with an electron capture detector (ECD) and Agilent HP-5 capillary column (30 m × 0.32 mm × 0.10 mm), organophosphorus pesticides nitrogen were determined with a phosphorus detector (NPD) and capillary Agilent HP-5 MSA column (30 m × 0.25 mm × 0.10 mm).

To determine mycotoxin (aflatoxin B1), a liquid chromatograph Agilent 1260 (Agilent, USA) with fluorometric detector (FLD) series G1321B and a capillary column Agilent Zorbax SB-C 18 (4.6 m × 150 mm × 5 μm) were used.

#### Laboratory Methods

The experiments were performed to meet the requirements for food safety set out in the documents: **ISO 22000 (2005)** Food safety management systems. Requirements for any food chain organizations; **ISO 22002-1 (2009)** Prerequisites for food safety; CAC/RCP 1-1969, Rev.4-2001

Recommended International Code of General Principles of Food Hygiene.

The total number of microorganisms (TNM) and the number of mold colonies in 1 m<sup>3</sup> of air were determined. Petri dishes with nutrient medium (NA) were used to determine (TNM) The cultures were incubated in a thermostat at a temperature of 30 ±0.5 °C for 72 hours (**DSTU ISO 4833, 2006**). Accounting for crop growth was carried out by the method of counting colonies per 1 m<sup>3</sup> of air by the formula (1):

$$x = \frac{y \times 10000}{78}, \quad (1)$$

Where:

x – is the total number of microorganisms, m<sup>3</sup>; y – the average number of colonies grown on five Petri dishes; 10000 – constant value; 78 – the area of the Petri dish, cm<sup>2</sup>.

Saburo agar with glucose was used to detect mold fungi. The cultures were incubated in a thermostat at a temperature of 22.5 ±0.5 °C for 5 days. The result was the average number of colonies grown on five Petri dishes.

Bactericidal properties of the disinfectant in the laboratory were determined according to the handbook **Kovalenko, Nychik and Mandyga (2014)**. Cleaning and disinfection and quality control were carried out following the guidelines **Paliy and Rodionova (2016)**.

#### Description of the Experiment

Nutrient media following (State Standard) **DSTU ISO/TS 11133-1 (2005)**, laboratory glassware, and laboratory equipment under (State Standard) **DSTU ISO 1042 (2005)** were used for research. The manufacture of solutions of reagents, paints, indicators, which were used for microbiological analysis, was carried out under (State Standard) **DSTU 5093 (2008)**.

Samples for the determination of heavy metals were prepared by the method of dry mineralization in accordance with **DSTU 7670 (2014)**. Lead, cadmium, copper, zinc, mercury were determined in accordance with **GOST 30178 (1996)** 'Raw material and food-stuffs. Atomic absorption method for determination of toxic elements'. Arsenic was determined according to **GOST 26930-86 (1987)** 'Raw material and food-stuffs. Method for determination of arsenic'.

Pesticides were determined by thin-layer chromatography approach (TLC): HCH (gamma isomer), DDT, DDD, DDE - according to MV No 2142-80 § 2.5-2.6 'Methodological instructions for the determination of organochlorine pesticides in water, food, feed, and tobacco products with thin layer chromatography', bazudin, carbophos, chlorophos, DDVP – in accordance with MV No 3222-85 Unified method for the determination of organophosphorus pesticides in the product of vegetable and animal origin, medicinal plants, water, soil by chromatographic methods'.

Determination of aflatoxin B1 was carried out according to MV No 4082-86 'Methodological instructions for detection: identification and determination of aflatoxins in food raw materials and food products by high-performance liquid chromatography'.

**Sample preparation:** The final determination of the bactericidal action of the disinfectant was performed on sterile test objects contaminated with microorganisms: strips of lawn fabric measuring 1 cm × 2 cm, wooden bars,

ceramic, metal, plastic, glass plates measuring 12 cm × 12 cm × 2 cm, which used in experiments repeatedly after sterilization in an autoclave (132 °C, 2 atm., 60 min). Sterile test items were placed in metal enameled cuvettes placed in a sterile box. A mixture of experimental test culture suspension and sterile bovine serum was applied to the test items. per 1 cm<sup>3</sup> of two billion suspension of microorganisms – 0.5 cm<sup>3</sup> of serum. The 1.5 cm<sup>3</sup> mixture is evenly distributed on the surface of each test object. The test culture is dried on test objects at room temperature for 24 hours.

**Number of samples analyzed:** The air of the chamber – 94.

**Number of repeated analyses:** 5

**Number of experiment replication:** 3

**Statistical Analysis**

Statistical processing of the obtained data was carried out on a computer system using spreadsheets Microsoft Excel XP Professional and STATISTICA 7.0 (Stat Soft, USA). It has been determined the arithmetic mean (M), the statistical error of the arithmetic mean (m). The probability of the difference between the arithmetic mean of the two variation

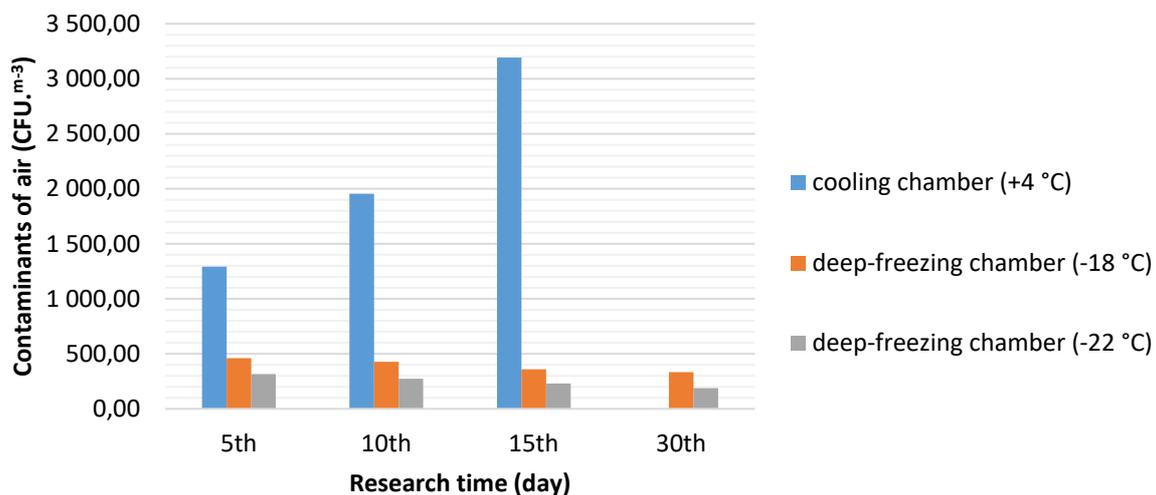
series was determined by the reliability criterion and Student’s tables.

**RESULTS AND DISCUSSION**

**Analysis of the sanitary-microbiological composition of air in refrigerators**

At the first stage of our research, we analyzed the sanitary-microbiological composition of the air of refrigeration chambers in the conditions of basic meat processing enterprises. During the study, the total number of microorganisms in the air (TNM) was determined. The results of the bacteriological examination are shown in Figure 1.

According to the results of research presented in Figure 1, it was found that the average number of microorganisms in the air of the cooling chambers (4 °C) varies from (12.92 ±0.4) × 10<sup>2</sup> CFU.m<sup>-3</sup> on the 5<sup>th</sup> day of storage of raw meat to (31.92 ±0.2) × 10<sup>2</sup> CFU.m<sup>-3</sup> at the end of the storage period (15 days). The obtained results indicate a proportional increase of TNM in cooling chambers during the storage of raw meat. On the 15<sup>th</sup> day of storage, this Figure is 2.5 times higher than on the 5<sup>th</sup> day.



**Figure 1** The total number of microorganisms in refrigerators, CFU.m<sup>-3</sup> (n = 5).

**Table 1** Results of sanitary-microbiological research of air of refrigerating chambers (n = 5, M ±m)

Air temperature °C	Research period	Results of bacteriological research of air, CFU.m <sup>-3</sup>				The average number of colonies on 1 cup
		Average number	Number of mold colonies on 5 Petri dishes including			
			Cladospores	Tamnidum	Penicillium	
+4	5 <sup>th</sup> day	10.6 ±1.3	0.4 ±0.2*	0	0.4 ±0.2*	2.1 ±0.3
-18		11.8 ±1	0.4 ±0.2*	0.4 ±0.2*	2.0 ±0.5*	2.4 ±0.2
-22		12.4 ±1	0.8 ±0.4*	0.8 ±0.4*	2.4 ±0.5*	2.5 ±0.2
+4	10 <sup>th</sup> day	20.6 ±1	3.6 ±0.9*	1.4 ±0.4*	7.0 ±0.3*	4.12 ±0.2
-18		17.2 ±1	0.6 ±0.2*	0.4 ±0.2*	4.2 ±0.7*	3.44 ±0.2
-22		19.8 ±0.7	0.8 ±0.4*	0.8 ±0.4*	5.0 ±0.3*	4.0 ±0.1
+4	15 <sup>th</sup> day	26.4 ±1.2	3.0 ±0.6*	1.3 ±0.2*	6.4 ±0.8*	5.3 ±0.2
-18		31.2 ±1.4	3.0 ±0.6*	1.2 ±0.5*	2.6 ±0.4*	6.2 ±0.3
-22		30.8 ±1.0	2.4 ±0.2*	1.6 ±0.2*	6.0 ±0.3*	6.2 ±0.2
-18	30 <sup>th</sup> day	34.2 ±1.1	3.6 ±0.5*	1.6 ±0.2*	3.0 ±0.7*	6.8 ±0.2
-22		36.6 ±1.99	2.0 ±0.32*	2.6 ±0.51*	6.4 ±0.75*	7.32 ±0.4

Notes: p >0.999 comparison of the value of the total number of molds and by species.

Analyzing the sanitary condition of deep freezing chambers No 1 (-18 °C) and No 2 (-22 °C), we came to the conclusion that during the shelf life of raw meat TNM decreases proportionally on average from  $(3.9 \pm 0.7) \times 10^2 \text{ CFU.m}^{-3}$  (5<sup>th</sup> day of storage) to  $(2.6 \pm 0.7) \times 10^2 \text{ CFU.m}^{-3}$  at the end of the storage period (30<sup>th</sup> day), in other words by 1.5 times (33.3%). It should be noted that the results of the study show that the lower the temperature in the deep freezer, the greater the bacteriostatic effect it demonstrates. Comparing TNM in deep-frozen chambers with a temperature of -22 °C and TNM in chambers with a temperature of -18 °C, it was found that on the 5<sup>th</sup> day of storage of raw meat this Figure is lower in chambers with a temperature of -18 °C by 31.4%, on the 10<sup>th</sup> day – by 36%, and by 43.6% – on the 30<sup>th</sup> day (end of the storage period).

Thus, the results of microbiological studies indicate a high degree of contamination of the air in the chambers of the refrigeration premises by microorganisms, which are a source of bacterial contamination of raw meat.

Analyzing the results of the research, a high positive correlation ( $r$ ) was found between the TNM in the air of the refrigeration chambers and the air temperature in the chamber. It was found that the correlation between TNM in the air of meat cooling chambers and TNM in the air of deep-freezing chambers (-18 °C) is equal to ( $r = 1$ ), while between TNM in the cooling chamber of meat raw materials and TNM in the deep freezing chamber (-22 °C) –  $r = 0.643$ .

During the technological process of cooling and freezing meat, special attention should be paid to the presence of microscopic mold fungi in the air, especially cladospores. This is because cladospores can show their toxigenic properties at low temperatures.

To determine the level of contamination of refrigerators with mold fungi, a sanitary-microbiological study of the air of the refrigeration chambers with different storage temperatures of raw meat was conducted. The results of the experiment are presented in Table 1.

Analyzing the materials in Table 1, the conclusion that the low temperature of refrigerators (from +4 °C to -22 °C) does not adversely affect the activity of mold fungi could be made. Microbiological examination revealed the following species of molds: *Tamnidium*, *Penicillium*, and *Cladosporium*.

It was found that the number of mold colonies in the cooling chambers (+4 °C) during 15 days of meat storage increases almost by 2.5 times.

It should be noted that in the cooling chamber at the end of the storage period of meat (15<sup>th</sup> day) bacterial air pollution by mold fungi was  $26.4 \pm 1.2 \text{ CFU.m}^{-3}$ , among them: fungi of the species *Cladosporium* – 4.9%, *Tamnidium* – 20.15% and *Penicillium* – 24.2%.

During the microbiological examination of the air in the deep-freeze chambers (-18 °C and -22 °C) during 30 days of storage of raw meat, the number of mold colonies increases almost by 3 times, reaching  $35.4 \pm 1.2 \text{ CFU.m}^{-3}$ .

The most contaminated with mold fungi on the 30<sup>th</sup> day of meat storage was the air of deep-freezing chambers at a temperature of -22 °C, where the total number of colonies of mold fungi was  $36.6 \pm 1.99 \text{ CFU.m}^{-3}$ , which is 6.5% more, than in the chamber at a temperature of -18 °C. It should be noted that among the total number of mold fungi in deep-frozen chambers at a temperature of -18 °C most often

recorded fungi of the species *Cladosporium* (10.5%), while in deep-frozen chambers at a temperature of -22 °C most often recorded fungi of the species *Penicillium* (17.5%).

It should also be noted that among the total number of identified colonies of molds ( $22.9 \pm 2.9 \text{ CFU.m}^{-3}$ ) in the chambers of the refrigeration shop, the percentage of fungi of the species *Cladosporium* was 8.3% ( $1.9 \pm 0.4 \text{ CFU.m}^{-3}$ ), *Penicillium* – 17.9% ( $4.1 \pm 0.7 \text{ CFU.m}^{-3}$ ), and *Tamnidium* – 4.8% ( $1.1 \pm 0.2 \text{ CFU.m}^{-3}$ ).

Our data correlate with the results of **Bogatko and Sakhniuk's studies (2013) who also** found that the technological modes of cooling meat in the cooling ( $t = -1$  °C) and refrigeration ( $t = -12$  °C) chambers do not have a bacteriostatic effect on the life of mold fungi. It was determined that the highest infestation of air fungi and plaster walls of the chambers is observed in their lower part, at a height of 0.5 m from the floor (**Bogatko and Sakhniuk, 2013**).

The results of our research are confirmed by the results of **Kukhtyn et al. (2020a)**. They have experimentally substantiated the quantitative indicators of the content of psychrotrophic microorganisms on the surface of beef intended for storage in a cooled and frozen state. Proved that the storage of meat in the frozen state inhibits or completely stops the development of mesophilic microorganisms for 20 days (**Kukhtyn et al., 2020a; Kukhtyn et al., 2020b**).

Thus, our research results indicate a satisfactory assessment of the sanitary condition of refrigeration chambers and deep-freeze chambers, and therefore require quality disinfection to remove mold colonies before the next laying of raw meat.

### Formulation development and testing of disinfectant

It should be noted that preventing the production, sale, and consumption of meat products, which are affected by pathogenic microorganisms and molds that can harm the health of the population, is one of the most important problems of meat processing plants. To address this issue, it is necessary to carry out systematic veterinary and sanitary control of refrigeration chambers, which includes sanitary and hygienic control of cleanliness and quality control of washing and disinfection.

Obligatory and timely cleaning and preventive disinfection of equipment and inventory used in the processing of raw meat are important elements of continuous industrial sanitary control, which includes sanitary and microbiological control of cleanliness, quality control of washing, and disinfection. Therefore, there is a need to find new universal, easy-to-use, safe disinfectants that, together with the availability and low cost, would be highly effective.

To sanitize the objects of veterinary control of meat processing enterprises, we have developed a recipe for a domestic disinfectant. The composition of the disinfectant includes hydrogen peroxide (8.0 – 10%), acetic acid (10%), peracetic acid (5.0 – 7.0%), stabilizing additives, water.

At the previous stage of the study, the spectrum of bactericidal action of the developed disinfectant against test cultures of microorganisms was studied (Table 2).

From the results presented in Table 2, it is seen that the developed disinfectant when used at a concentration of 0.01% at an exposure of 10 – 60 min, at a concentration of 0.03% at an exposure of 10 – 30 min and a concentration of 0.05% at an exposure of 10 min does not show bactericidal action to test cultures of *E. coli*, *S. Dublin* and *S. aureus* microorganisms. When using the drug at a concentration of 0.03% at an exposure of 60 min, at a concentration of 0.05% at an exposure of 30 min, and a concentration of 0.1% at an exposure of 10 min, it was found that it destroys test cultures of *E. coli* and *S. Dublin*, however, it acts bacteriostatically on *S. aureus*. The bactericidal action of this disinfectant was determined when used at a concentration of 0.05% at an exposure of 60 min, at a concentration of 0.1% at an exposure of 30 – 60 min, and at a concentration of 0.15% at an exposure of 10 min.

The obtained research results gave us a basis for further testing of the developed disinfectant. The next step was to conduct experiments involving test objects contaminated with test cultures of microorganisms. Sterile bovine serum was used as biological protection to determine the effect of organic contaminants on the bactericidal activity of the disinfectant. The results of the studies are presented in Table 3.

From the materials presented in Table 3, it is seen that the disinfectant, which has been developed, when it is used at a concentration of 0.03% at an exposure of 10 – 60 min does not show a disinfectant effect on these test objects.

**Table 2** The results of the study of bactericidal properties of the disinfectant (n = 3).

Concentration, %	Exposure, min	Test culture of microorganisms		
		<i>E. coli</i>	<i>S. Dublin</i>	<i>S. aureus</i>
0.01	10	+	+	+
	30	+	+	+
	60	+	+	+
0.03	10	+	+	+
	30	+	+	+
	60	-	-	+
0.05	10	+	+	+
	30	-	-	+
	60	-	-	-
0.10	10	-	-	+
	30	-	-	-
	60	-	-	-
0.15	10	-	-	-
	30	-	-	-
	60	-	-	-
control	10	+	+	+
	30	+	+	+
	60	+	+	+

Notes: «-» - growth of microorganisms is absent; "+" - the growth of microorganisms is available.

**Table 3** The results of the study of the disinfectant action of the disinfectant (n = 3).

Test culture	Lawn		Wood			Tile			Metal			Plastic			Glass			
	Concentration, min																	
	10	30	60	10	30	60	10	30	60	10	30	60	10	30	60	10	30	60
concentration 0.03%																		
<i>E. coli</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>S. Dublin</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>S. aureus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
concentration 0.05%																		
<i>E. coli</i>	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
<i>S. Dublin</i>	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
<i>S. aureus</i>	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
concentration 0.10%																		
<i>E. coli</i>	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
<i>S. Dublin</i>	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
<i>S. aureus</i>	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
concentration 0.15%																		
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. Dublin</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Notes: «-» - there is no growth of microorganisms; "+" - the growth of microorganisms is available.

**Table 4** Activity of disinfectant in production conditions (n = 3).

Object disinfection	Regime application	Samples were investigated			% disinfection
		total	including		
			disinfected	not disinfected	
metal	0.05%, 60 min	60	60	–	100
plastic		60	60	–	100
glass		60	60	–	100
metal	0.1%, 30 min	60	60	–	100
plastic		60	60	–	100
glass		60	60	–	100
metal	0.15%, 10 min	80	80	–	100
plastic		80	80	–	100
glass		80	80	–	100

**Table 5** Results of toxicological studies of meat after storage.

Name of indicator and unit of measurement	MDR according to regulatory documents	Research results *		
		cooling chamber (+4 °C)	deep-freezing chamber (-18 °C)	deep-freezing chamber (-22 °C)
<b>Toxic elements</b>				
Mass fraction of lead, mg.kg <sup>-1</sup>	Not more 0.5	0.12456	0.32043	0.12452
Mass fraction of cadmium, mg.kg <sup>-1</sup>	Not more 0.05	0.00652	0.00234	0.00186
Mass fraction of copper, mg.kg <sup>-1</sup>	Not more 5.0	1.03450	0.87445	1.05602
Mass fraction of zinc, mg.kg <sup>-1</sup>	Not more 70.0	10.81625	13.88760	10.62380
Mass fraction of arsenic, mg.kg <sup>-1</sup>	Not more 0.1	Not detected (less sensitivity of the method <0.02)	Not detected (less than the sensitivity of the method <0.014)	Not detected (less than the sensitivity of the method <0.014))
Mass fraction of mercury, mg.kg <sup>-1</sup>	Not more 0.03	Not detected (less sensitivity of the method <0.00155)	Not detected (less sensitivity of the method <0.00185)	Not detected (less sensitivity of the method <0.00255)
<b>Pesticides</b>				
HCH (gamma isomer), mg.kg <sup>-1</sup>	Not more 0.1	Not detected <0.05	Not detected <0.05	Not detected <0.05
Mass fraction of bazudin	Not allowed	Not detected <0.2	Not detected <0.2	Not detected <0.2
Mass fraction of carbophos	Not allowed	Not detected <0.2	Not detected <0.2	Not detected <0.2
Mass fraction of chlorophos	Not allowed	Not detected <0.2	Not detected <0.2	Not detected <0.2
Mass share of DDVP	Not allowed	Not detected <0.2	Not detected <0.2	Not detected <0.2
Mass fraction of DDT	Not more 0.1	Not detected <0.05	Not detected <0.05	Not detected <0.05
Mass share of DDD	Not more 0.1	Not detected <0.05	Not detected <0.05	Not detected <0.05
Mass fraction of DDE	Not more 0.1	Not detected <0.05	Not detected <0.05	Not detected <0.05
Mass fate of metaphor	Not allowed	Not detected <0.2	Not detected <0.2	Not detected <0.2
<b>Mycotoxins</b>				
Mass concentration of aflatoxin B1	0.005	Not detected (less method sensitivity <0.001)		

Note: \* – the competence of the laboratory for these methods is confirmed by NAAU in accordance with State standard **DSTU ISO/IEC 17025 (2006)**.

At the action of the drug at a concentration of 0.05% and 0.1% at exposures of 60 and 30 min, respectively, and a concentration of 0.15% at 10 min, it was found that it destroys test cultures of microorganisms, regardless of the treated test-object. These ways of application of the drug were tested in production conditions.

To assess the sanitary condition of the meat processing plant in the washings after disinfection, the presence of the amount of MAFAnM and *Escherichia coli* bacteria was determined. For this purpose, we used the method of direct seeding on nutrient media. The results of the experiment are presented in Table 4.

According to the results presented in Table 4, it was found that the developed disinfectant at a concentration of 0.05% (60 min), 0.1% (30 min) and 0.15% (10 min) completely disinfects surfaces made of different materials (metal, plastic, glass), and the use of the drug in these modes allowed 100% disinfection of objects of veterinary control of meat processing enterprises.

To study the effect of disinfectants on the safety of meat, a toxicological study of raw meat was performed 5 days after placing in the freezer. Product samples were taken following the Procedure for sampling products of animal, plant, and biotechnological origin for research, approved by

the Resolution of the Cabinet of Ministers of Ukraine of June 14, 2002, No 833 (CMU, 2002).

According to the results of toxicological studies of frozen beef (Table 5) on the 5<sup>th</sup> day of storage in the refrigeration chambers for the presence of toxic elements (lead, cadmium, zinc, arsenic, mercury), pesticides, and mycotoxin (aflatoxin B1) deviations from the norm are not detected. The data obtained show that there is no effect of residual disinfectant on raw meat.

Disinfection is a major component of the general set of veterinary and sanitary measures that ensure a high level of sanitation in the meat processing industry (Fagerlund et al., 2017; Coughlan et al., 2016; Møretrø, Langsrud and Heir, 2013; Larsen et al., 2014).

For this purpose, several disinfectants from different groups of chemical compounds are used (Da Costa Luciano et al., 2016; Salata et al., 2018; Paliy et al., 2018). However, not all disinfectants are effective and efficient. This is due to the high organic contamination of the treated surfaces (Hultman et al., 2015; Zwirzitz et al., 2020), the low bactericidal activity of disinfectants, the formation of resistance in microorganisms to chemical compounds (Saá Ibusquiza, Herrera and Cabo, 2011; Ortiz et al., 2015; Møretrø et al., 2017).

To expand the range of antimicrobial agents, we have developed a formula of an innovative disinfectant, which includes environmentally friendly compounds such as hydrogen peroxide, acetic and peracetic acids. The use of these compounds for disinfection has become widespread in many countries around the world (Hawley et al., 2018; Stopiglia et al., 2011).

The obtained results are expanding the range of highly active antimicrobial drugs that are promising during their use in the food industry. The proposed disinfectant is effective import-substituting development.

Decomposing only to oxygen and water, hydrogen peroxide is one of the environmentally safe and affordable chemicals (Pędzwiatr et al., 2018). And organic acids are even used for food disinfection (Wang et al., 2019). Therefore, these advantages of hydrogen peroxide and organic acids led to our choice to design a disinfectant formula for use in the meat industry. During testing, a disinfectant was developed in the laboratory and its modes of application were established to disinfect test objects (lawn, wood, tile, metal, plastic, glass) contaminated with test cultures of microorganisms (*E. coli*, *S. Dublin*, *S. aureus*). Our chosen methodological approaches to the assessment of disinfectants correlate with the reports of other researchers (Tomasino, 2013; Tishyn et al., 2017; Rabenau et al., 2020). In assessing the disinfectant, it is necessary to model the production conditions (Becker et al., 2019), which were taken into account by us. The established modes of application of disinfectant were tested in production conditions, which gave grounds to recommend this tool for widespread use in the processing industry.

## CONCLUSION

It was found that the average number of microorganisms in the air of the cooling chambers (+4 °C) increases proportionally during storage of raw meat from  $(12.92 \pm 0.4) \times 10^2$  CFU.m<sup>-3</sup> on the 5th day of storage to  $(31.92 \pm 0.2) \times 10^2$  CFU.m<sup>-3</sup> at the end of the storage period (15<sup>th</sup> day). The

lower the temperature in the deep-freeze chambers, the greater the bacteriostatic effect on microorganisms.

Among the total number of identified colonies of molds ( $22.9 \pm 2.9$  CFU.m<sup>-3</sup>) in the chambers of the refrigeration shop, the percentage of fungi of the species *Cladosporium* was 8.3% ( $1.9 \pm 0.4$  CFU.m<sup>-3</sup>), *Penicillium* – 17.9% ( $4.1 \pm 0.7$  CFU.m<sup>-3</sup>), and *Tamnidium* – 4.8% ( $1.1 \pm 0.2$  CFU.m<sup>-3</sup>). It is proved that the low-temperature regime of refrigeration chambers (from +4 °C to -22 °C) does not have a detrimental effect on the activity of mold fungi.

Developed disinfectant (hydrogen peroxide (8.0 – 10%), acetic acid (10%), peracetic acid (5.0 – 7.0%), stabilizing additives, water) provides destruction of sanitary-indicative microorganisms in refrigerators meat processing plants when used in a concentration of 0.05% – 60 min, 0.1% – 30 min, 0.15% – 10 min and has no toxic effect on raw meat, which is stored in the chambers of the refrigeration plant after disinfection.

Strict observance of the requirements of technological processes of meat storage and processing and high-quality disinfection ensure satisfactory sanitary and hygienic conditions of cooling and refrigerating chambers.

Prospects for further research are to provide theoretical and experimental justification for the use of new detergents and disinfectants in the meat processing industry and their impact on the final product, as well as to establish the features of legal support of proper sanitary and hygienic requirements of the refrigeration chain in Ukraine and meat processing industry compared to the requirements of the European Union.

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#### Conflict of Interest:

The authors declare no conflict of interest.

**Ethical Statement:**

This article does not contain any studies that would require an ethical statement.

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