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DISINFECTION OF MEAT INDUSTRY EQUIPMENT AND PRODUCTION ROOMS WITH THE USE OF LIQUIDS CONTAINING SILVER NANO-PARTICLES

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Abstract: Essential sorts of disinfecting agents and principles of their action have been considered in the paper. Results of research on application of washing-disinfecting liquids, containing silver nano-particles, in meat industry have been presented. It has been established that liquids characterized by very efficient bactericidal and fungicidal properties can be prepared by composition of toxically performing silver nano-particles, some degreasing agents, reducing surface tension, and frothing agents. The listed components eliminate "insulating effect" of fat particles in which bacteria are suspended and facilitate silver nano-particle contact with bacteria and fungi, increasing this way biochemical effect of silver nano-particles.

INTRODUCTION

Biocides are applied in various industries in order to reduce population of hazardous microorganisms harmful to health of human beings or animals as well as those affecting quality of food products in meat industry [3, 13].

According to UE directive [2], regulations concerning biocidal products [15] and law regulating categories towards their objectives [12], the biocidal products are defined as "active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means". According to the above regulations [12] biocidal substances can be divided into 23 groups in 4 categories. The products of the 1st category, including disinfecting and bactericidal products of general application, are presented in Table 1.

Microorganisms, after entering the animal- or human-organism can cause numerous infectious diseases. They can be transferred from the environment by atmospheric air, water or food products, contaminated carrier or by contact with contaminated surface [1, 14]. In food industry a very good medium for microorganism growth is created by residual pieces of food left in technological machines. As a result, fresh food subjected to technological process may undergo microbiological recontamination [10]. Probability of food contamination and human or animal organism infection is low when the environment of technological line is kept properly clean. Cleanliness, adequate to the require-

Table 1. Classification of bactericidal products of the 1st category [3, 12]

Category	Group of biocidal products	
Disinfecting and biocidal products of general application	1	Biocidal products used for personal hygiene
	2	Disinfecting products for personal and public use and other biocidal products
	3	Biocidal products used for veterinarian hygiene
	4	Products used for disinfection of surfaces that come into contact with food and animal
	5	Products applied for water, used by people and animals, disinfection

ments of technological process can be ensured by application of washing and disinfection processes. The aim of these processes is removing of the mechanical, chemical and microbiological impurities from the surface of machines, appliances, packing materials and also from production compartments [7, 10]. Washing and disinfecting process has been presented in Figure 1.

In the washing process mechanical and chemical impurities are removed, a however,

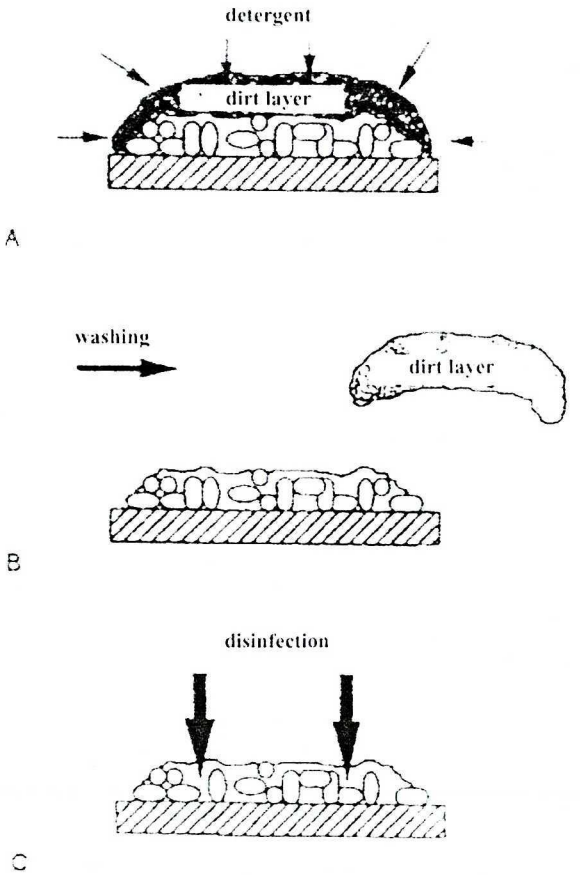

Fig. 1. Stages of washing and disinfection
A – detergent dissolves organic and inorganic components of the dirt, B – washing, separation of dirt layers, uncover the microorganisms in biofilm, C – disinfection (penetration into biofilm and inactivation of microorganisms [19]

number of microorganisms is reduced only from the level of 10^6 to the level of 10^3. A disinfection process can reduce the microorganism's population down to the level of 10^1 [19]. Disinfection kills vegetative forms of microorganism by using chemicals or applying physical methods [7]. The most frequently used methods, are based on the application of chemical agents.

In the present work results on application of disinfecting, containing silver nanoparticles alkali liquids for meat production compartments, smoke chambers, technological machines and appliances in meat industry disinfection have been presented.

DISINFECTING METHODS AND DISINFECTING AGENTS

Distribution of a disinfecting agent on the disinfected surface can be achieved by using "cold steam", foaming or spraying method. [4]. Fog, made by "cold steam" method, covers all surfaces uniformly making a film of active substance. Foaming method enables precise observation of the disinfecting sites and longer exposure time to disinfectant. Spraying method is the most widespread method consisting in preparation of working solution for the disinfection. Disinfecting agents are selected according to the aim, range, and application place [7, 19]. Chemical disinfectants should be characterized by efficient bactericidal action towards hazardous microorganisms; however, they should not induce immunity mechanisms in bacteria, viruses or fungi. Disinfecting agents, by wetting the surface perfectly, should act fast, efficiently even at low concentrations used, and be not toxic for the people and animals. They should also be water soluble, biodegradable and not inducing corrosion [19]. Bactericidal effect of chemical agent depends mainly on the sort and number of undesirable microorganisms, concentration and time of exposure to the chemical, and also on the environmental factors such as temperature, pH, humidity and organic substance [7, 18].

Disinfecting agents affect microorganisms (viruses, bacteria, fungi) by damaging their cytoplasm and causing protein denaturation and degradation of nucleic acids. Hydro-sulphur (-SH) groups- elements of protein structure- undergo oxidation that results in malfunctioning of their system [7, 11, 19]. Concentration of the active substance determines the effectiveness of the disinfecting agent, that can be bactericidal (irreversible structural and metabolic changes in cells) or biostatic (inhibited growth of microorganisms).

Viruses are microorganisms of a transitory form between simple protein molecules and bacteria. Such primitive structures consist of nucleic acid sheathed with proteins [1, 8, 16]. They contain genetic material (DNA, RNA), however, they cannot metabolize independently, so, they use for this purpose cells of a host organism causing its disease.

Bacterium cell is built of nucleus, where genetic material is located and protoplasm closed within cell membrane, adjacent to the rigid wall, impervious for high-molecular compounds. Cell membrane, built of lipids and proteins is permeable for metabolic compounds [1, 8, 16]. A large number of bacteria form endospore enclosed in thick membrane. Under favorable conditions spores transform into vegetative cells [8]. Fungus cell, containing glycogen, fats and dye is enclosed in chitin membrane. Fungi cells feed on products resulted from organic matter decomposition [8, 16].

The most important disinfecting compounds are quaternary ammonium compounds, peroxides, Cl- and J- containing compounds, alcohols, aldehydes, bactericides, phenols, cresols and heavy metal salts [6, 7, 10, 18, 19].

Quaternary ammonium compounds perform disinfecting, wetting and emulsifying functions. Preparations containing ammonium salts affect Gram-positive, Gram-negative, yeast, fungi, and viruses. Their action consists in attraction of salt cation to negatively charged surface of the microorganism cell. That decreases wall permeability of the cell and hinders transport of feeding components to cytoplasm. Cation can also penetrate to cytoplasm, where, through enzyme inactivation inhibits metabolism of the cell. The disadvantage of the agents containing quaternary ammonium compounds is formation of immune to their action microorganisms. To counteract, an alternate disinfecting agents are to be applied. Some limitations in the quaternary ammonium compounds application can also result from their inactivation by such substances as protein, fat or blood.

Compounds, such as peracetic acid, hydrogen peroxide, potassium persulphate are able to damage vegetative forms of bacteria as well as bacterial spore. The most widely used preparations, containing the above compounds, are mixtures of peracetic acid, hydrogen peroxide and water. The compounds used, oxidize the SH-groups in proteins into disulphide forms, causing malfunctioning of membrane and wall of the microorganism cell. Particular advantage of peroxides is their biodegradability.

Cl-containing compounds are strong oxidants and effectively destroy microorganisms. This group includes chloramine T, and sodium hypochlorite. Effectiveness of the sodium hypochlorite is based on its hydrolysis resulting in hypochlorous acid formation. The latter, after penetration into microorganisms, destroys bacteria, fungi und viruses. Ionized oxygen, released in the process, denaturizes proteins, inactivates enzymes and oxidize -SH groups. Chlorine concentration in solutions, used for disinfection purpose, should be on the level of 100 to 200 ppm. Bactericidal effect of chlorine can be reduced by the presence of organic compounds in the environment.

Iodophors are complex compounds of iodine with polymers, biopolymers or surface active compounds. Iodine, released from iodophors, inhibits activity of respiration enzymes of microorganisms through oxidation of hydro-sulphur groups and through complexing of proteins of cell membrane. Iodophors affect all microorganisms at concentrations of active form as low as 12–25 ppm.

Alcohol containing disinfecting agents cause denaturation of protein cells of microorganisms. The highest effects are achieved when alcohol concentration is ~ 65%. The most important for disinfection are ethyl- and propyl alcohol. Alcohols, however, should be applied to the surfaces previously washed.

Aldehyde containing agents, because of their toxicity, can be applied only to walls and floors disinfection. An example is preparation of the mixture of formalin of the concentration of 3–20%. It is a strong bactericidal agent, destroying also bacterial spore.

Bactericides are amphoteric organic compounds, for example, aminoacids with substituted groups. They display acidic or alkaline properties, depending on pH of the environment.

Phenol and its derivatives destroy vegetative forms of bacteria and fungi; however, they do not affect much bacterial spore. At present, the phenol is not applied in disinfecting preparations. Instead, biphenols, cresols, xylen phenols are used. They react with

structural proteins of microorganism cells consequently inactivate cell wall and membrane. Phenols, due to their toxic properties, are not in application.

Among heavy metal salts, applied for disinfection, are silver and mercury salts. They react with -SH structural groups of proteins and enzymes negatively affecting metabolism of microorganisms. Particular recognition was received by nitrate, citrate and lactate of silver. However, solutions containing colloidal metallic silver are applied the most. Such solution, containing nano-particles of metallic character, displays stronger biochemical activity.

Disinfecting chemicals can destroy microbiological source of impurities or make it harmless. Nevertheless, the microbiological organisms can recuperate when the environmental conditions change [9]. Favorable conditions for microorganism growth can be reduced by regular washing and disinfection. The very important factor is, however, application of disinfectants, containing different sorts of active substances.

The basic problem in food industry is disinfection of production rooms and production lines in order to prevent the growth of different kind of bacteria, and mould fungi. For this purpose, different kinds of agents have been applied for a very long time. Historically, the first conserving and disinfecting agents applied were vegetable extracts from horseradish, garlic, onion, nettle, or acetic acid solution. Peracetic acid solution has been one of the most frequently used bactericidal agent until now. Application of calcium hydroxide in the form of lime is also frequently used as a bactericidal and fungicidal agent for household room disinfection and for tree lime whiting.

Modern disinfecting and bactericidal agents usually contain several, typical for washing, components such as surface actives, emulsifiers, complexones, anti-flocculants, foaming and other components with different proportions, depending on the application purpose. The main purpose of these mixtures is to create physico-chemical conditions enabling effective bactericidal action of alkali, particularly sodium hydroxide – the main component of all disinfecting solutions.

Among the newest bactericidal substances nano-silver in water solution can be distinguished. Bactericidal properties of nano-silver have been known since ancient times, when silver cups for drinking water were used to protect from infection [17]. The ability to kill bacteria, moulds, fungi and bacterial spores by silver compounds were already known at the beginning of the 19th century, however the mechanisms of silver action had not been described. Nowadays, it is known that toxic for the microorganisms silver ion can affect directly an individual cell. Owing to catalytic properties silver oxidizes genetic material of the cell.

Water solution, containing nano-particles of metallic silver of 10^{-12} m in large deposited on different carriers, for example, silica or polymer, is one of the modern disinfecting and bactericidal agents. Tests on its application for technological line and industrial compartments disinfection gave positive results. However, there was fear that concentration of silver used (300 ppm) might be toxic to the environment and that its application could be expensive.

The presence of fats hinders bacteria and fungi contact with silver particles and restrain the oxygen access to the silver surface resulting in a decrease of disinfecting effects.

EXPERIMENTAL PART

The aim of the work was preparation of solutions, enabling to create the most effective bactericidal and fungicidal properties of silver and its compounds. Simultaneously, the silver concentrations should not be at a toxic level and technological procedure should be economically profitable. That is particularly important for meat, food and related industries, where the problem comes from fatty surfaces, creating perfect conditions for bacteria and fungi of the mould group growth.

The liquids manufactured for this purpose should be characterized by the properties listed below.

- Ability to reduce surface tension at the interface between the washing liquid and dirty (fatty) surface. This requires an introduction of a surface-tension agent into the disinfecting liquid.
- Ability to form complex compounds with metal ions present as impurities. This requires application of adequate complexing agents.
- Ability to prevent secondary precipitation of dirt. This requires application of emulsifiers.
- Ability to ensure permanent foaming by addition of foam stabilizer in order to increase components interaction. This is particularly important when the washed surface has places not easily accessible.
- Ability to ensure alkali medium helping in dissolving fats. This requires sodium hydroxide addition.
- The content of nano-silver should be adequate to ensure its efficient action without threat of toxic effect of the metal.
- Disinfected elements of technological appliances and rooms should meet the European requirements, according to permitted number of microorganisms TVC, (0–10/cm^2), and *Enterobacteriae* (0–1/cm^2).

In the present work formula for several disinfecting liquids has been worked out. 100 kg of concentrate K1 characterized by bactericidal, washing-disinfecting, non-foaming properties of alkaline liquid was prepared using 0.5 kg of anionic surface active agent in the form of 86.5% solution of alkyl ether of carboxylic acid (trade name Akyl LF4); 15 kg of sodium hydroxide solution (50% NaOH); 1 kg of complexing agent in the form of solution, containing 24% of hexasodium salt of diethyl triamine-penta (trade name Dequest 2066); 1 kg of the agent that prevents secondary precipitation of dirt, in the form of solution containing 45% of sodium acrylate and aliphatic aminoacid (trade name Sokalan PA 30); 0.5 kg of nano-composite, containing 2000 ppm of Ag in the form of particles ~ 10^{-12} m deposited on silica carrier (trade name Nano Silver) and 82 kg of water.

Concentrate K2 of bactericidal, washing-disinfecting, and foaming characteristics in the amount of 100 kg of alkaline liquid was prepared using 5 kg of anionic surface agent of emulsifying and dissolving properties in the form of alkyl ether of carboxylic acid (trade name AKYPO RLM 45CA); 5 kg of sodium hydroxide (50% NaOH), 3 kg of the agent that prevents secondary precipitation of dirt, in the form of solution containing 45% of sodium acrylate and aliphatic aminoacid (trade name Sokalan PA 30); 10 kg of foam stabilizer in the form of oxide of amine alkyl dimethyl coconut oil (trade name Oxidet DMC LD); 16 kg of nano-composite, containing 2000 ppm of Ag in the form of particles ~ 10^{-12} m deposited on silica carrier (trade name Nano Silver) and 59 kg of water.

Working solutions of different concentrations were prepared by diluting the K1 and K2 concentrates as below:

- 100 kg of concentrate K1 was diluted, under continuous stirring, by addition of 400, 900 and 1900 kg of water.
- 100 kg of concentrate K2 was diluted, under continuous stirring, by addition of 900, 2900 and 4900 kg of water.

In the next stage of work three new agents, containing silver nano-composite were investigated:

- Alkaline washing-disinfecting agent (CLIX K.01) appropriated for washing and cleaning smoke-curing chambers with help of foam-making equipment. It is a highly alkaline and foaming agent. Its composition: alkali, detergents, dispersants, stabilizers, silver nano-composite. Application parameters: concentration: 3–5.0%, temperature: 60–80°C, time of exposure 10–20 minutes.
- Alkaline washing-disinfecting agent (CLIX M.01) appropriated for washing machines, walls and floors with use of foam. It is a highly alkaline agent efficiently removing organic impurities, such as fat and proteins. Its composition: dispersants, alkali, detergents, silver nano-composite. Application parameters: concentration 3–4.0%, temperature: 40–50°C, time of exposure 10–20 minutes.
- Alkaline washing-disinfecting agent (CIP-CLIX P.01) appropriated for washing plastic containers. It is a not-foaming alkaline agent. It removes heavy dirt of organic nature. Its composition: alkali, detergents, sequestration agent, dispersants, silver nano-composite. Application parameters: concentration 0.5–1.0%, temperature: 30–55°C, time of exposure 5–10 minutes.

The solutions obtained by diluting the concentrate K1 and K2 were applied for washing of four technological systems in meat industry. After washing, means of two measurements of total number of microorganisms TVC (standard is 0–10/cm^2) and number of bacteria of *Enterobacteriae* group (standard 0–1/cm^2) were obtained. The objects were analyzed according to PN-ISO 18593:2005 and the results obtained (Tab. 2) were interpreted according to [5].

From Table 2, it can be concluded that working solutions prepared by dilution of the concentrates K1 and K2 meet the standards concerning the total number (0–10/cm^2) of microorganism TVC and number of *Enterobacteriae* (0–1/cm^2).

Tests on application of alkaline foaming liquid CLIX K.01 for washing smoke chamber gave satisfactory washing and foaming results. Stable, slowly sliding down the

Table 2. Results of analysis, (mean of two measurements) concerning the total number of microorganism TVC (standard: 0–10/cm^2) and number of *Enterobacteriae* (standard: 0–1/cm^2), in the working solutions

Liquid applied	Number of TVC microorganisms/cm^2	Number of microorganisms from *Enterobacteriae* group/cm^2
K1 (1:4)	0.0	0.0
K1 (1:9)	0.4	0.0
K1 (1:19)	2.0	0.25
K2 (1:9)	0.0	0.0
K2 (1:29)	0.0	0.0
K2 (1:49)	0.5	0.25

wall foam accumulated dirt, fat and tar from the surface. The liquid applied contained 4% of concentrate in water of the temperature ~ 20°C. The temperature inside the smoke chamber before the washing started was 60°C; contacting time of foam with washing surface was ~ 15 minutes.

Tests on application of alkaline foaming liquid CLIX M.01 for washing and disinfecting surfaces of the machines and installations gave satisfactory results. Stable, slowly sliding down the surface foam accumulated dirt and fat. The liquid applied contained 3% of concentrate in water of the temperature ~ 45°C. Contacting time of foam with washing surface was ~ 15 minutes.

Satisfactory results were also obtained using alkaline liquid CLIX P.01 for washing containers. Microbiological smear tests provided results within the standard requirements for plastic materials. The concentrate was diluted with water of 55°C to yield concentration of 0.5%. Contacting time of washing solution with washed surface was ~ 15 minutes

CONCLUSIONS

The formula of two concentrates K1 and K2 and several disinfecting liquids was been worked out experimentally.

It has been established that liquids obtained with use of concentrates K1, K2 and those with symbols CLIX K.01, CLIX M.01, CLIX P.01 are characterized by very efficient bactericidal and fungicidal properties. These properties are enhanced by addition of silver nano-particles (toxically affecting bacteria), some degreasing agents, reducing surface tension and foaming agents. The listed components eliminate "insulating effect" of fat particles in which bacteria are suspended, and facilitate access of nano-silver particles to bacteria and fungi. This results in an increase of biochemical effect of nano-silver. Additional effects consist in removing particles of dust and other impurities from washed surfaces.

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ZASTOSOWANIE PŁYNÓW ZAWIERAJĄCYCH NANO-CZĄSTECZKI SREBRA DO DEZYNFEKCJI URZĄDZEŃ I POMIESZCZEŃ PRODUKCYJNYCH W PRZEMYŚLE MIĘSNYM

W pracy przeanalizowano podstawowe rodzaje środków dezynfekcyjnych i zasady ich działania. Przedstawiono wyniki badań zastosowania płynów myjąco-dezynfekujących zawierających nano-cząsteczki srebra w przemyśle mięsnym. Stwierdzono, że można otrzymać płyny o doskonałych własnościach bakteriobójczych i grzybobójczych w kompozycjach z dodatkiem nano-cząsteczek srebra działających toksycznie na bakterie oraz odpowiednich środków odtłuszczających, zmniejszających napięcie powierzchniowe i spieniających. Składniki te eliminują „izolacyjne" działanie tłuszczu, w którym zawieszone są bakterie i rozwijając powierzchnię kontaktu cząsteczek srebra z bakteriami i grzybami zwiększają oddziaływanie biochemiczne nano-cząsteczek srebra.