UDC 637.5.033:637.5.037:664.951:637.07

STUDY OF USE OF ANTISEPTIC ICE OF PLASMA-CHEMICALLY ACTIVATED AQUEOUS SOLUTIONS FOR THE STORAGE OF FOOD RAW MATERIALS

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DOI: https://doi.org/10.15673/fst.v15i4.2260

Article history

Received 03.07.2021
Reviewed 18.08.2021
Revised 24.10.2021
Approved 01.12.2021

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Cite as Vancouver style citation

Pivovarov O, Kovalova O, Koshulko V. Study of use of antiseptic ice of plasma-chemically activated aqueous solutions for the storage of food raw materials. Food science and technology. 2021;15(4):95-105. DOI: https://10.15673/fst.v15i4.2260

Цитування згідно ДСТУ 8302:2015

Pivovarov O., Kovalova O., Koshulko V. Study of use of antiseptic ice of plasma-chemically activated aqueous solutions for the storage of food raw materials // Food science and technology. 2021. Vol. 15, Issue 4. P. 95-105. DOI: https://doi.org/10.15673/fst.v15i4.2260

Abstract. Scientists and specialists of food processing industry are increasingly focused on the technology of storage of raw materials of animal origin in antiseptic ice. The paper presents the peculiar features of plasma-chemical activation of solutions to produce antiseptic ice and their further use in the storage of meat and fish. The process of treatment leads to formation of micro-particles of hydrogen peroxide, which in contact with the raw materials are capable of generating active oxygen, allowing to disinfect the raw material surface. Concentration of hydrogen peroxide in the solutions further used to produce antiseptic ice has been determined. Study of the features of use of antiseptic ice made of plasma-chemically activated aqueous solutions showed unchanged organoleptic properties of raw materials after using the presented antiseptic agent. Suppression of viable microflora owing to antimicrobial action of plasma-chemically activated aqueous solutions was recorded. Antiseptic ice contributes to long-term disinfection of raw materials. For example, the samples show absence of microflora of mesophilic aerobic and facultative anaerobic microorganisms and bacteria of the Escherichia coli group, which allows improving the quality of stored food raw materials. At the concentration of peroxides at the level of 600–700 mg/l, pathogenic microflora in meat and fish samples is not present at all. When meat and fish raw materials are stored in ice for 30 days, pathogenic microflora does not appear, and it confirms the long-term disinfecting action of antiseptic ice. Slower accumulation of amino-ammonia nitrogen is observed during long-term storage of meat and fish raw materials. The use of plasma-chemically activated aqueous solutions for the production of antiseptic ice and storage of meat and fish raw materials in it can partially prevent the processes of protein breakdown and, accordingly, increase the product shelf life. The paper describes technological parameters of the process of storage of meat and fish in antiseptic ice, which can be used in the industrial storage of raw materials, and provides the guidance for use of ice of plasma-chemically activated aqueous solutions in the process of storage of raw materials of animal origin.

Key words: plasma-chemical activation, aqueous solutions, hydrogen peroxide, antiseptic ice, meat, fish.

Introduction. Formulation of the problem

Modern technologies for the storage of fish and meat involve the use of low temperatures. However, it is impossible to improve the process of food raw materials' storage by varying the temperature parameters only. Currently, the innovation methods, which would provide sufficiently long shelf life, depending on a large number of contributing factors, are being sought. Bacterial contamination with various microorganisms is one of the most common problems during storage. The modern food industry employs a large number of novel approaches to improve the safety of fresh meat and fish: freezing with antibiotics added, preparation of crushed ice, obtaining of electrochemically activated solutions followed by mixing with raw materials, obtaining of ice of the frozen activated water using millimeter-range electromagnetic radiation, obtaining of antiseptic ice from acidified aqueous ozone solutions and so on [1]. Absolutely all methods have their strengths and weaknesses. For example, application of antibiotics is rather expensive method having a negative impact on the consumer health.

At present time, the use of water treated by electro-physical methods of influence in meat and fish industries is a promising area, which solves a number of pressing technological problems. Aqueous solutions activated in different ways are used in many industries, in particular, in food production. Study of changes in the properties of water and aqueous solutions in the process of activation can expand the diversity of techniques in food production. Certain properties of the activated aqueous solutions can improve quality and safety of food products [2-4]. The use of ice made of specially prepared aqueous solutions is of great interest to scientists and industrial operators, since the introduction of such preserving material can improve the process of long-term storage of food raw materials without addition of harmful chemicals.

Analysis of recent research and publications

The technology of storage of food raw materials stipulates the preservative effect of low temperatures, but it is not enough to ensure long-term storage, which depends on a variety of related factors. Bacterial contamination with pathogenic microorganisms is one of the main causes of rapid spoilage of food products and raw materials [1,5]. Currently, a number of new methods to increase safety of fresh meat and fish are used. The use of various techniques for cooling of meat in storage in the food industry is the best way to prevent or slow down the spoilage of these products. Cold treatment neutralize the activity of microorganisms and suppresses the chemical and biochemical processes occurring in the product under the action of enzymes, atmospheric oxygen, heat and light. The depth and nature of changes in raw materials during cooling and storage depend on the derived parameters of the product, i.e. type and quality of raw materials, conditions, modes of storage, antiseptic treatment and other factors of influence [6]. Physico-chemical methods used for processing of raw materials and solutions for their storage can significantly improve the existing technologies.

Particular attention in the innovative technologies is paid to the use of effects of low-temperature plasma on the chemical and physico-chemical processes in the environments under study. Low-temperature plasma is an advanced technology attracting a lot of attention due to its ability to disinfect food raw materials of various origins [7]. Plasma-activated water contains different high-reactive forms of oxygen and reactive forms of nitrogen. Much attention is given to the introduction of plasma-chemical technologies to improve biological and chemical safety of food products [7]. Plasma-chemically treated water has its effect on food product quality (chemical, physical and sensor properties). Plasma-chemically treated aqueous solutions are used as a medium for thawing and preserving of products in combination with the other traditional processing methods. The mechanism of microbial inactivation by plasma-ctivated water is divided into three main stages (Fig.1), including destruction of the cell membrane and intracellular components and release of intracellular materials and components [7].

The difference in the technology of use of low-temperature plasma [7] is the presence of free forms of active nitrogen, which may have unpredictable effect on the processed raw materials.

Ice cooling is one of the most commonly used methods for the food product storage under the influence of low temperature, since it can delay the development of bacterial contamination [8]. Production of safe edible ice featuring universal antimicrobial properties is important for the safety of food products and consumer health and it has been of great interest in recent years. The results of the latest technological research conducted by G. Katsaros indicate the possibility of use of the activated ice containing bactericidal substances as a potential disinfectant for the storage of raw food products such as fresh vegetables, seafood and various fish products [9].

Treatment of aqueous solutions with cold atmospheric plasma and acid electrolysis of water represent two technologies proposed in [9] for treatment of water, which became the basis for ice production. In addition, scientists [9] proposed to use ultraviolet LEDs to treat ice by irradiation.

Cold plasma is one of the most promising methods for the food industry since it involves minimal processing. However, until recently, limited studies reported on the effects of cold plasma on processed seafood. Scientists Soni A., Choi J., Brightwell G. recorded the positive effect of plasma treatment onto microbiological quality, physico-chemical and sensory properties and oxidation rate of processed fish and seafood and other food raw materials [10-11]. Moreover, cold plasma can also be used as a method of food processors' wastewater treatment [2].

The effect of electrolyzed water ice, compared to the traditional ice obtained from tap water, on the microbiological and chemical indicators of quality of fish (Pacific saury) has been recorded. Ice is prepared with the addition of active chlorine at the concentration of 34 mg/kg of weakly acidic electrolyzed water. Microbiological analysis showed that this ice significantly inhibited the growth of bacteria in the saury pulp during its storage in the refrigerator, primarily due to the action of active chlorine. Chemical analysis proved that ice under study slowed down the generation of volatile basic nitrogen and substances reacting with thiobarbituric acid, and reduced the accumulation of alkaline compounds in the fish meat compared to ordinary ice. Organoleptic analysis confirmed that saury stayed fresh 4-5 days longer compared to fish stored with the addition of ice made of ordinary water [12].

To produce antiseptic ice, a number of agents are commonly used. For example, extracts of plants such as thyme (Thymus serpyllum) have a positive effect on the preservation of fish raw materials. Stable preservative effect of such ice has been proved [13].

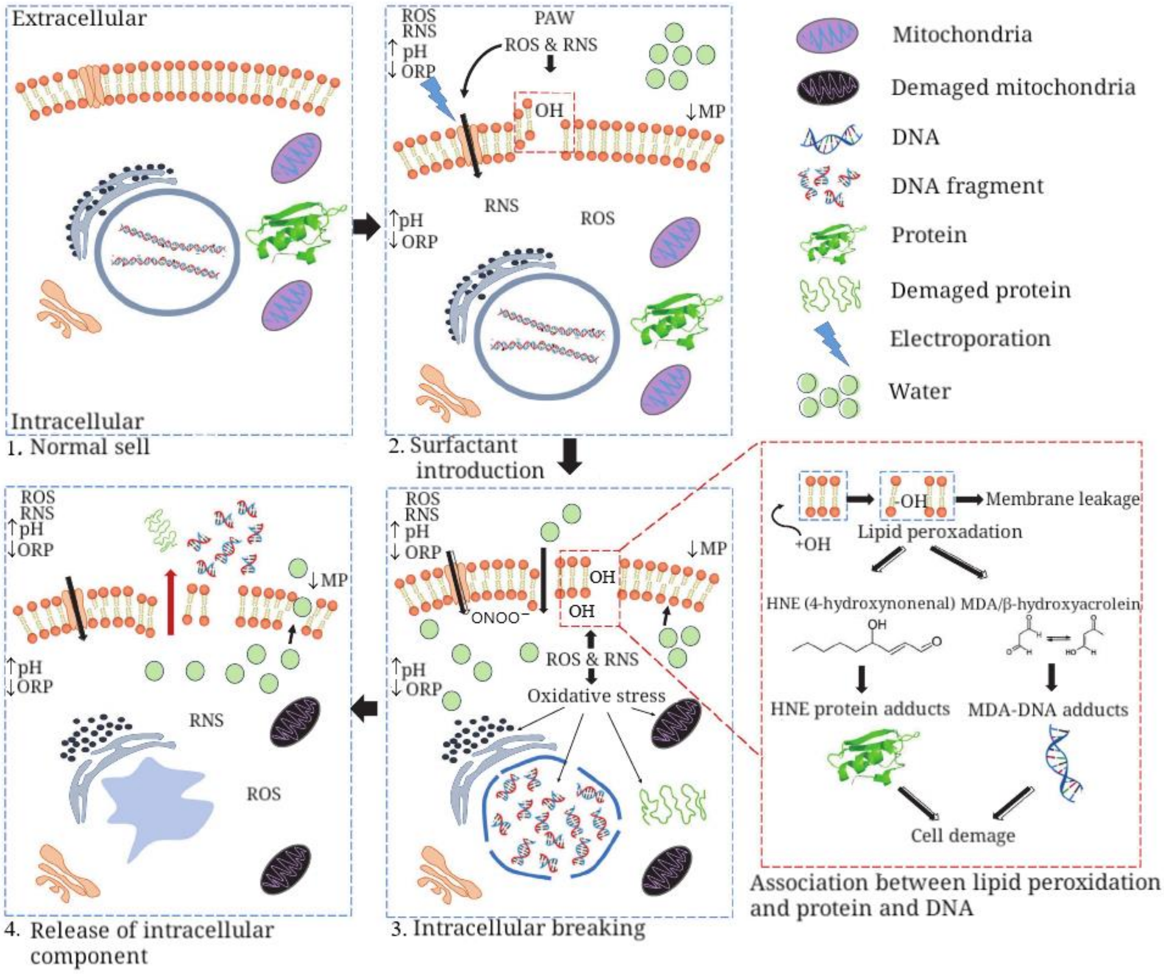

Fig. 1. Mechanism of microbial inactivation by plasma-activated water [7]

PAW – plasma-activated water; ROS – highly reactive oxygen species; RNS – reactive nitrogen species; DNA – deoxyribonucleic acid; pH – potential of hydrogen; ORP – oxidation-reduction potential; HNE – 4-hydroxynonenal, forms protein adducts during oxidative stress; MDA – malondialdehyde DNA

The products, which could slow down the microbial damage and lipid oxidation, are being sought. To ensure high-quality long-term storage of fish, the effect of chemicals used alone or in combination in the traditional water ice glazing was studied. Nisin, chitosan, phytic acid and combinations thereof were added to water used for freezing. Inclusion of natural chemicals in the ice glaze could prevent microbial spoilage and oxidation of lipids and, therefore, preserve the freshness of saury when stored frozen. Studies have shown that combined treatment with natural chemicals could be used commercially to preserve freshness and increase shelf life of fish [14]. It allowed using the proposed technology for industrial glazing of fish.

An important aspect of research is the use of activated water ice in the preservation treatment of shrimps. This ice showed significant advantage for inhibition of microbial growth, extending the shrimp storage time by 4–8 days. The pH of shrimps treated with ice remained below 7.7 during storage. Besides, changes in color characteristics and hardness of shrimps were delayed by the ice treatment, and protein composition of the product remained unchanged. All this confirms the viability of using activated water ice in preservation of fresh seafood [15].

Possibility of plasma technology use for the treatment of chicken breasts was studied as well [16]. Conclusions were made on the effective use of this technology to improve the microbiological safety of chicken breasts, leading to minor changes in sensory properties of the product. For example, pronounced disinfecting action of the activated water, especially towards pathogenic microflora, such as pathogens of salmonellosis, was recorded, as well as minor organoleptic changes in the raw material, namely, change in the meat color.

All these studies allow us to speak of the prospects of using plasma-chemical activation of aqueous solutions. Plasma-chemical activation of water and aqueous solutions is the first step to usage of the properties of water without its forced treatment with foreign chemicals. All processes during activation take place directly in water without addition of any foreign chemical components. Reactogenic properties of the activated water are of great interest to scientists, since the properties of water arising after activation can be a starting point in the development of the new area of nanotechnology. Water activated under the action of non-equilibrium contact plasma exhibits antiseptic and antibacterial properties. However, it should be noted that this water being a cluster structure after plasma treatment may have some new properties, previously little studied, but interesting from a practical standpoint [17]. The resulting activated water has a specific composition. The reaction products determining the reactivity of this water are the most easily detectable ones. This mainly applies to hydrogen peroxide and superoxide compounds, excited particles and radicals, which play an important role in redox processes [18-20]. It should also be noted that such water after plasma treatment may exhibit some new properties, previously little studied [21]. A special role in this case is given to the study of influence of activated water on the parameters of some processes in food, biochemical and biotechnological industries [22]. When it comes to the final components of water and aqueous solutions after treatment with non-equilibrium contact plasma, they can be represented as a mixture of hydrogen peroxide, superoxide components and active radicals and particles [23]. However, their quantitative characteristics (mkg and parts per million) cannot cause damage to human health. Such components act in several ways.

Plasma-chemically activated aqueous solutions have a specific composition: they contain hydrogen peroxide and superoxide compounds, excited particles and radicals, which play an important role in redox processes. Hydrogen peroxide is an antiseptic; getting into cells under the action of enzymes, it breaks down into water and oxygen having antimicrobial action, but no harmful chemicals remain in the cells. That is, products of redox reactions in contact with food products are converted into substances being a part of aqueous solutions before their plasma-chemical activation.

The phenomenon of activation of the aqueous solutions causes a number of specific physical and chemical effects [23-28], which may serve as starting points for advanced technologies. The use of plasma-chemical activation in many cases facilitates the production and reduces its cost, taking into account energy costs and time for activation. The shelf life of standard plasma-chemically activated aqueous solution is 6 months from the date of production under conditions of storage in closed containers.

Therefore, the further study of use of plasma-chemical activation of aqueous solutions with their subsequent freezing and production of antiseptic ice is an important area of research. The crucial but insufficiently explored aspect is the study of effect of plasma-chemically activated aqueous solutions on the microbiological parameters of raw materials stored for a long time.

The purpose of this work consists in studying of the effectiveness of plasma-chemically activated aqueous solutions used as antiseptic ice in the storage of meat and fish.

Objectives of the work:

1. Study of the effect of antiseptic ice from plasma-chemically activated aqueous solutions on the change in organoleptic indicators of meat and fish after long-term storage.
2. Study of the modes of plasma-chemical activation for the maximum disinfection of raw materials and extension of shelf life and preservation of marketable condition of fish and meat raw materials.
3. Study of the content of amino-ammonia nitrogen during storage of raw materials in antiseptic ice.

Research materials and methods

Raw materials taken for research are as follows: meat (chicken fillet, pork carbonade, and beef tenderloin), fish (common carp). To determine the effect of antiseptic ice on the organoleptic indicators of fish and meat raw materials, samples were subjected to cooling and storage for 5–30 days in ice.

For the experiments, we used antiseptic ice obtained from plasma-chemically activated aqueous solutions [23]; they differed in the concentration of peroxides (300–700 mg/l) in the derived activated solutions. Water activation was performed with the use of the laboratory plasma-chemical unit (Fig. 2).

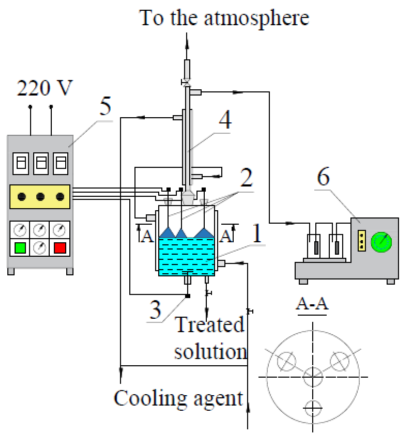

Fig. 2. Diagram of the laboratory three-arc plasma-chemical unit:
1 – reactor; 2 - anodes; 3 – cathode; 4 – reflux condenser; 5 – power supply; 6 – vacuum pump

Tap water was activated in plasma discharges of reduced pressure (0.08 KPa) with the voltage of 1000-1200 V and current of 30.0–200.0 mA (the current was changed in steps of 20 mA) with the subsequent transition to the mode of non-equilibrium contact plasma of 400 to 600 V voltage and current of up to 150 mA, according to the electrical conductivity increase.

Hydrogen peroxide content was determined by permanganatometry method based on the principle of oxidation of hydrogen peroxide with the strong oxidant (potassium permanganate) to free oxygen.

In order to establish the most effective method of storage, the methods of meat and fish processing such as cooling and storage in pieces of ice of different sizes (meat, fish) were used. Meat was cooled to the temperature from 0 to +4°C, and fish from –1 to +5°C; after that the pieces of ice obtained from activated aqueous solution were added to the raw materials. Microbiological studies of raw materials at different stages of storage were performed as well.

Ice scales of the temperature varying within –(6–12)°C (the coldest form of ice) were used as the ice. This form of ice is very dry, with the residual moisture content of about 2%, and looks like broken glass. The fraction of 1–2 mm was used for meat and fish. In large hypermarkets, ice thickness of 2.0–2.2 mm is used to display fish, and for seafood and meat display it is 1–1.2 mm. Ice scales was obtained using ЛГ-250Ч-01 ice generator designed for scale ice production. It is used for making of semi-finished products and sausages, as well as storage of vegetables, fruits, meat, fish and other raw materials.

The fish was mixed in trays with antiseptic ice obtained from plasma-chemically activated aqueous solutions (with or without addition of salt) with peroxide concentration of 300–700 mg/l. Ice temperature during storage varied within f –(6–12)°C. The products were stored in the laboratory in a special refrigerator.

Chicken meat, pork and beef were mixed with the bactericidal ice, packed and stored at the temperature of –(0–2)°C in the refrigerator where the specified temperature was maintained.

Microbiological indicators of raw materials were determined using conventional procedure. Microbial count of QMAFAnM was determined according to DSTU 8446:2015 by comparison of the effectiveness of bactericidal ice action on samples of raw materials cooled and stored in ice scales of tap water (control sample) and plasma-chemically activated aqueous solutions (experimental samples). After that, QMAFAnM was determined. To determine the indicators, Petri dishes were washed in accordance with the aseptic rules.

We determined the coliform bacteria on the surface of raw materials, conducting the research in compliance with the standard methods of DSTU 8381:2015. They were based on the inoculation of a certain amount of product on the liquid selective medium containing lactose to determine the fermentation properties of acid and gas generation and, whenever necessary, re-inoculation of the enrichment culture on the surface of special dense agar media, to confirm the affiliation of the isolated colonies with coliform bacteria by their cultural and biochemical characteristics.

The amino-ammonia nitrogen content was determined by titration using phenolphthalein. The method is based on the ability of water-soluble nitrogen-containing non-protein compounds of meat (amines, ammonium salts, amino acids, etc.) to be neutralized by alkali, which in the presence of the indicator gives a certain coloring [1].

Results of the research and their discussion

Antiseptic ice produced by freezing of aqueous solutions differs in the fact that it freezes plasma-chemically activated aqueous solutions at the temperature of –(5–40)°C. Characteristic of the activated water used for the production of antiseptic ice is given in Table 1.

Table 1 – Characteristics of water activated by non-equilibrium contact plasma

Experiment	Water	Activation time, min	Concentration of hydrogen peroxide, mg/l
1 (control)	Tap water	-	-
2	Activated water	10	300
3	Activated water	20	400
2	Activated water	30	600
4	Activated water	40	650
5	Activated water	60	700

Concentration of peroxides in the solutions depends on the time of their processing and ranges from 300 to 700 mg/l. The longer the water is activated in the reactor, the higher the peroxide content in the solution.

Plasma-chemically activated aqueous solutions were frozen in special block molds or ice generator at the temperature of –(5–40)°C. The ЛГ-250Ч-01 ice generator allowed obtaining ice of up to 1-2 mm; this fraction is generally accepted as optimal one for storing raw materials.

Further processing of the food product took place by its storing (possibly by mixing) in ice scales obtained on the basis of plasma-chemically activated aqueous solutions.

At the initial stage, the effect of antiseptic ice on the change in organoleptic indicators of meat and fish was studied. Antiseptic ice was taken with the maximum peroxide concentration of 700 mg/l (sample No.5), so it was possible to identify any violations of organoleptic indicators of raw materials, which could lead to melting of plasma-chemically activated solutions. It is found that with the use of proposed antiseptic agent organoleptic properties of the raw materials were unchanged, as evidenced by the results in Table 2.

Table 2 – Organoleptic indicators of raw materials after the use of antiseptic ice

Indicator	Raw materials			
	Meat		Fish	
Before the experiment: Color	Red color typical for muscle tissue (chicken meat – pink)		Grey color typical for skin cover	
Odor	Typical for meat raw materials		Typical for fish raw materials	
After cooling with the addition of ice	Control	Experiment No.5 (storage in antiseptic ice)	Control	Experiment No.5 (storage in antiseptic ice)
Color				
1 day	typical	typical	typical	typical
5 days	typical	typical	typical	typical
10 days	color change	typical	color change	typical
15 days	color change	typical	color change	typical
30 days	color change	typical	color change	typical
Odor				
1 day	typical	typical	typical	typical
5 days	typical	typical	typical	typical
10 days	odor change	typical	color change	typical
15 days	odor change	typical	color change	typical
30 days	odor change	typical	color change	typical

When we analyze the data of Table 2, it should be noted that owing to use of antiseptic ice obtained from plasma-chemically activated aqueous solutions, there was almost no unpleasant odor typical for meat and fish raw materials. With the use of antiseptic ice, the color remained unchanged for the long period, namely, for 30 days.

After receiving of the results of organoleptic evaluation, microbiological analysis was performed. It should be noted that resistance of modern strains of microorganisms to disinfectants and growth of their antibiotic resistance requires the development of new disinfectants [29-30].

The use of antiseptic ice of plasma-chemically activated aqueous solutions is aimed at increase in the shelf life of food products (meat and fish) [32] and suppression of viable microflora [31], causing damage to products, without the use of chemicals and antibiotics, due to antimicrobial action of aqueous solutions formed during melting of bactericidal ice and possible decrease in its melting point. Results of the microbiological study of the sanitary condition of fish and meat are given in Tables 3-4. The experiments were performed three times; the tables show the average result.

The use of antiseptic ice significantly reduces the microbial contamination of the product during its shelf life, and the product shelf life increases by 4-5 times. This is due to reduction of the number of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM) on the surface of the fish body (Table 3). For example, at the peroxide concentration of 600-700 mg/l, complete absence of microorganisms was observed, indicating the pronounced disinfectant properties of antiseptic ice. At lower concentrations, the remains of microorganisms were detected, but with the low dynamics to increase their number during storage, compared to the control sample. Similar dynamics was observed in the study of meat. No smell was recorded in the experimental samples before the end of the experiment.

The use of antiseptic ice of plasma-chemically activated aqueous solutions increases the shelf life of chicken meat 2-4 times owing to reduction of total number of microorganisms on the surface of chicken meat (Table 4). Pathogenic microflora dies under the impact of pathogenic microflora. Moreover, the optimal concentration of hydrogen peroxide, which is critical for the microflora in all experiments, is constant, indicating the possibility of using antiseptic ice with such hydrogen peroxide indicators for the large commercial batches of raw materials.

Table 3 – Study of microbiological condition (QMAFAnM) of fish when stored in antiseptic ice obtained from plasma-chemically activated aqueous solutions, CFU/g (n=3)

Days of storage	Control	Concentration of peroxides in plasma-chemically activated aqueous solutions, mg/l						
		100	200	300	400	500	600	700
Fish								
1	$3.1*10^2$	$2.5*10^2$	$1.0*10^2$	<10	0	0	0	0
5	$6.2*10^4$	$6.1*10^2$	$1.3*10^2$	<10	0	0	0	0
10	$4.5*10^5$	$1.1*10^3$	$1.7*10^2$	<10	0	0	0	0
15	$5.3*10^7$	$4.3*10^3$	$2.3*10^3$	$1*10^2$	0	0	0	0
20	$8.7*10^9$	$1.3*10^4$	$3.5*10^4$	$2*10^2$	0	0	0	0
25	$9.3*10^{11}$	$2.3*10^4$	$5.1*10^5$	$4*10^2$	<10	0	0	0
30	$3.1*10^{12}$	$5.3*10^4$	$6.7*10^5$	$5*10^2$	<10	<10	0	0

Table 4 – Study of microbiological condition (QMAFAnM) of meat when stored in antiseptic ice obtained from plasma-chemically activated aqueous solutions, CFU/g (n = 3)

Days of storage	Control	Concentration of peroxides in plasma-chemically activated aqueous solutions, mg/l						
		100	200	300	400	500	600	700
Chicken meat								
1	$4.3*10^2$	$1.6*10^2$	$1.0*10^2$	<10	0	0	0	0
5	$8.6*10^4$	$1.0*10^3$	$1.3*10^2$	<10	0	0	0	0
10	$4.3*10^5$	$5.4*10^3$	$1.7*10^2$	<10	0	0	0	0
15	$9.3*10^7$	$4.6*10^4$	$2.3*10^3$	$1*10^2$	0	0	0	0
20	$5.7*10^9$	$9.1*10^4$	$3.5*10^4$	$2*10^2$	<10	0	0	0
25	$7.3*10^{10}$	$5.4*10^4$	$5.1*10^5$	$4*10^2$	<10	<10	0	0
30	$7.1*10^{12}$	$9.3*10^4$	$6.7*10^5$	$5*10^2$	<10	<10	0	0
Pork								
1	$3.6*10^2$	$2.1*10^2$	$1.3*10^2$	<10	0	0	0	0
5	$7.3*10^4$	$2.4*10^3$	$2.4*10^2$	<10	0	0	0	0
10	$3.2*10^5$	$3.6*10^3$	$5.1*10^2$	<10	0	0	0	0
15	$6.1*10^7$	$5.4*10^4$	$3.4*10^3$	$2*10^2$	0	0	0	0
20	$8.4*10^9$	$7.3*10^4$	$6.1*10^4$	$3*10^2$	<10	0	0	0
25	$6.1*10^{11}$	$8.5*10^4$	$7.7*10^5$	$5*10^2$	<10	<10	0	0
30	$4.5*10^{14}$	$6.4*10^4$	$8.5*10^5$	$7*10^2$	<10	<10	0	0
Beef								
1	$7.1*10^2$	$2.3*10^2$	$1.4*10^2$	<10	0	0	0	0
5	$6.5*10^4$	$1.5*10^3$	$3.3*10^2$	<10	0	0	0	0
10	$5.7*10^5$	$4.4*10^3$	$6.5*10^2$	<10	0	0	0	0
15	$8.6*10^7$	$6.1*10^4$	$1.2*10^3$	$2*10^2$	0	0	0	0
20	$9.1*10^9$	$5.8*10^4$	$7.1*10^4$	$3*10^2$	<10	0	0	0
25	$4.7*10^{10}$	$7.4*10^4$	$6.8*10^5$	$5*10^2$	<10	<10	0	0
30	$5.4*10^{14}$	$8.2*10^4$	$8.7*10^5$	$6*10^2$	<10	<10	0	0

When we analyze the dynamics of changes in the microflora in the meat samples under study, the pronounced disinfectant effect should be noted. Quality of meat raw materials during storage depends both on the sanitary-and-hygienic condition of the environment and the degree of its initial microbiological contamination [6]. In the process of raw materials' storage in the refrigerated state, the number of microorganisms is changed. For example, the amount of microflora in the control gradually increases, since at low temperatures the development of mesophilic microorganisms on the meat surface is stopped. With regard to experimental samples stored in antiseptic ice, inhibition of microflora was observed with the increase in concentration of peroxides in the solutions. For example, after reaching of peroxide concentration of 600 mg/l, the existing pathogenic microflora was completely neutralized and surface of the raw material became conditionally sterile. Moreover, stable antiseptic effect was maintained throughout the period of experiment, as evidenced by the results shown in Tables 3-4.

Bacteria of the Escherichia coli group do not die during cooling; as a rule, they do not cause the meat spoilage, but may result in food poisoning [6]. Their presence in samples was monitored, and results are given in Table 5.

When we analyze the results given in Table 5, it is necessary to note the effectiveness of the proposed antiseptic ice in disinfection of raw materials from E.coli. For example, all samples are fully decontaminated from coliform bacteria at the peroxide concentration of 400 mg/l, indicating good perspectives for using this ice in the storage of fish and meat raw materials. In addition, long-term antiseptic effect of ice was observed during storage for 30 days. It is necessary to note the increased sensitivity of coliform bacteria to antiseptic ice based on plasma-chemically activated solutions, namely, E.coli on the surface of raw materials under study are completely inactivated. However, we observe pronounced resistance of bacteria of the Escherichia coli group to low concentrations of peroxides: at the concentration of 100–200 mg/l coliform bacteria do not undergo any changes, even after prolonged contact with ice. Nevertheless, even at the concentration of 300 mg/l we can see the antiseptic effect of ice at the 10-15th day of storage, but this is not enough for high-quality preservation of raw materials. Pronounced antiseptic effect is observed at the concentration of 400 mg/l. Besides, consistency of this result is recorded throughout the storage period.

This antimicrobial effect is explained by the fact that activated aqueous solutions, as mentioned above, contain hydrogen peroxide and superoxide compounds. Hydrogen peroxide is a common classical antiseptic; getting into cells under the action of enzymes (peroxidase and catalase), it breaks down into water and oxygen having antimicrobial action, but no harmful chemicals remain in the cells, so there is no chemical contamination of food raw materials. Plasma-chemically activated aqueous solutions do not bring unwanted odors and tastes and allow not to use chemical preservatives [33].

Table 5 – Study of presence of coliform bacteria in meat and fish when stored in antiseptic ice obtained from plasma-chemically activated aqueous solutions (in 0.001 g) (n = 3)

Days	Control	Concentration of peroxides in plasma-chemically activated aqueous solutions, mg/l						
		100	200	300	400	500	600	700
Chicken meat								
1	+	+	+	+	–	–	–	–
5	+	+	+	+	–	–	–	–
10	+	+	+	–	–	–	–	–
15	+	+	–	–	–	–	–	–
20	+	+	–	–	–	–	–	–
25	+	+	–	–	–	–	–	–
30	+	+	–	–	–	–	–	–
Pork								
1	+	+	+	+	–	–	–	–
5	+	+	+	+	–	–	–	–
10	+	+	+	+	–	–	–	–
15	+	+	+	–	–	–	–	–
20	+	+	–	–	–	–	–	–
25	+	+	–	–	–	–	–	–
30	+	+	–	–	–	–	–	–
Beef								
1	+	+	+	+	–	–	–	–
5	+	+	+	–	–	–	–	–
10	+	+	–	–	–	–	–	–
15	+	+	–	–	–	–	–	–
20	+	+	–	–	–	–	–	–
25	+	+	–	–	–	–	–	–
30	+	+	–	–	–	–	–	–
Fish								
1	+	+	+	+	–	–	–	–
5	+	+	+	+	–	–	–	–
10	+	+	+	–	–	–	–	–
15	+	+	+	–	–	–	–	–
20	+	+	–	–	–	–	–	–
25	+	+	–	–	–	–	–	–
30	+	+	–	–	–	–	–	–

Note: “+” – found; “-” – not found.

One of the possible mechanisms of action of the activated aqueous solutions on bacteria is the change in outer layers of the cell, which makes receptors accessible for reactogenic enzymes, such as lysozyme. Free radicals form a gap in the cell wall, causing the loss of selective permeability. Hydrogen peroxide as a part of activated aqueous solutions causes the destruction of surface structures and internal membranes in microorganisms [34]. The integrity of cytoplasmic membrane disrupts a number of membrane-related enzymes, such as dehydrogenases, and reduces the efficiency of DNA repair systems. Bactericidal activity of hydrogen peroxide and activated aqueous solutions is connected, primarily, with high oxidative capacity and action of toxic products occurring during lipid peroxidation. Peroxide oxidation affects ribosome proteins, causing their destruction. Besides, destruction of membrane structures is facilitated by the formation of peroxide compounds. The action of hydrogen peroxide or activated water causes local destruction of the integral cell wall and disruption of the permeability of bacterial cells during the first minutes of contact. However, low concentrations of peroxides may not have an immediate effect, i.e. a small dose of peroxides in ice takes a long time for starting to act on the microflora. Consequently, peroxides as a result of gradual melting of ice will accumulate on the surface and show the expected disinfecting effect over time. However, the time spent can have a negative impact on the raw materials, so it is rational to use ice with the concentration of peroxides being critical for coliform bacteria, i.e. at least 400 mg/l.

During storage of meat and fish raw materials, protein substances are broken down under the action of proteolytic enzymes of bacteria to produce ammonia compounds in the form of its salts and free amino acids, the amount of which can indicate the product freshness or spoilage. Therefore, we analyzed the changes in this indicator during storage in all presented samples of raw materials. The results are shown in Fig. 3. Changes in the indicator were recorded from the first day of storage.

When we analyze the data of Fig. 3, it is necessary to note that despite complete suppression of the vital activity of the microflora with the use of some experimental ice samples, growth of amino-ammonia nitrogen still occurs. This is due to the fact that the decay processes continue but at a slower rate, and even with complete sterility it is not possible to completely stop the destructive processes in the tissues and muscles.

Dynamics of changes in the content of amino-ammonia nitrogen in the control and experimental samples during storage is shown in Fig. 3. The studies indicate the increase in this indicator during storage. It is the expected result because proteins are broken down in the process of storage. However, we should say that during storage of raw materials in antiseptic ice of plasma-chemically activated aqueous solutions, accumulation of amino-ammonia nitrogen is slower at the concentration of peroxides in antiseptic ice varying from 300 to 700 mg/l. That is, protein breakdown occurs more intensely in samples stored in ice made of ordinary tap water. The use of plasma-chemically activated aqueous solutions for the production of antiseptic ice and storage of meat and fish raw materials can partially prevent the processes of protein breakdown and, accordingly, increase shelf life of the product, as evidenced by the research results. It allows us to speak of the possibility of long-term storage of the raw materials under study.

The use of plasma-chemically activated aqueous solutions maintains the microbiological stability of food raw materials during storage, completely replacing the traditional antiseptic agents, food acids and acidifiers. Therefore, the presented technology for using antiseptic ice in the industrial production can be reasonably introduced and may be of interest to the processing industry and retail chains in the processes of cooling and storage of meat and fish.

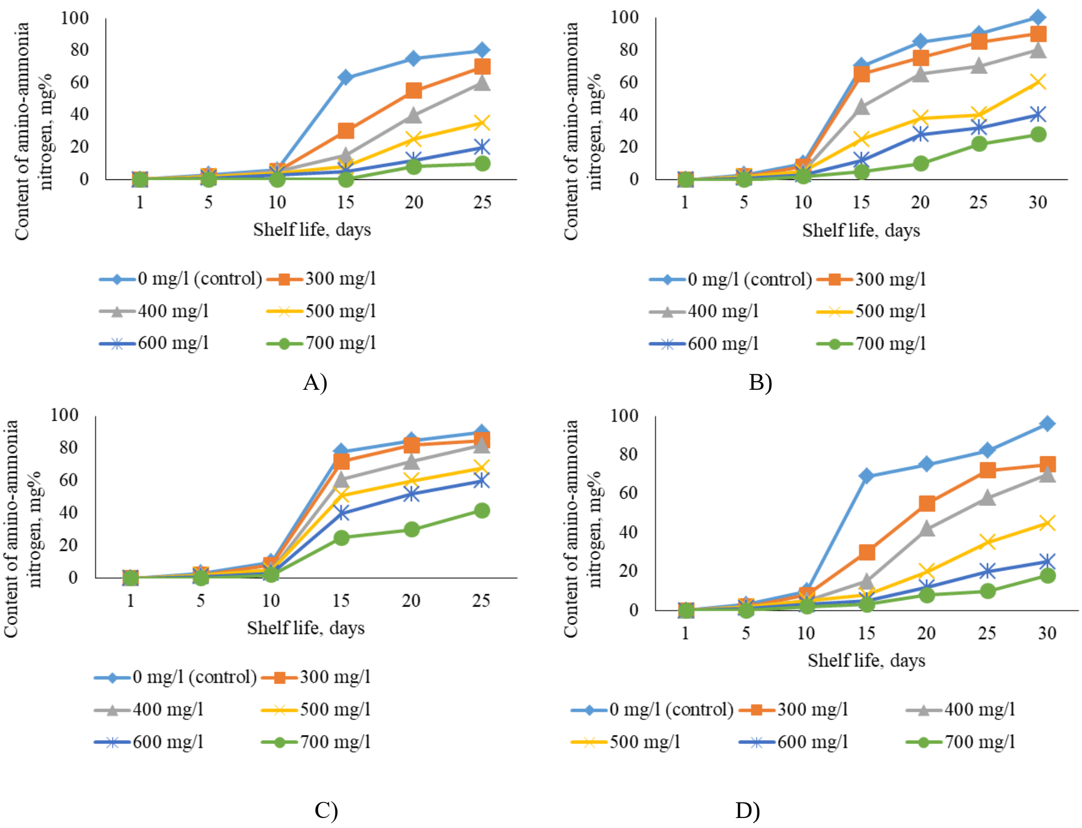

Fig. 3. Effect of concentration of peroxides of plasma-activated aqueous solutions during storage on the content of amino-ammonia nitrogen in samples: A – chicken meat; B – pork; C – beef; D – fish.

Consequently, the use of plasma-chemically activated aqueous solutions in the preparation of bactericidal ice can significantly increase the shelf life of food products from 10-15 to 30 days without the use of chemicals and pharmaceutical agents by significantly reducing the amount of microflora on the product surface in contact with water formed during melting of antiseptic (bactericidal) ice. Bactericidal ice obtained by freezing of plasma-chemically activated aqueous solutions has the reduced melting point due to presence of free active radicals; it can maintain the low temperature of the raw materials for longer. For example, antiseptic ice with the temperature of –(6–12)°C is capable of maintaining stable temperature indicators of raw materials for the minimum 15 days on average, instead of 10.

Results of the work show that antiseptic ice obtained from plasma-chemically activated aqueous solutions increases the shelf life of products of animal origin, without compromising the environmental safety of the products themselves and the environment.

Conclusions

1. We analyzed the effect of antiseptic ice of plasma-chemically activated aqueous solutions with the hydrogen peroxide concentration of 300–700 mg/l on the change in organoleptic indicators of meat and fish after long-term storage. In accordance with our results, with the use of antiseptic ice there was almost no unpleasant odor typical for meat and fish raw materials, and the color remained unchanged for 30 days.

2. We determined the most effective modes of plasma-chemical activation for the maximum disinfection of raw materials and extension of shelf life, that is, concentration of peroxides in solutions used for ice production ranged from 300 mg/l and more. Accordingly, the complete destruction of microflora at the stage of processing and subsequent storage was observed at peroxide concentration of 600 mg/l.

3. Study of the microbiological condition of raw materials during storage with the use of antiseptic ice of plasma-chemically activated aqueous solutions allowed us to speak of provision of the product microbial safety. The use of ice with peroxide concentration of 600–700 mg/l allowed obtaining completely decontaminated raw materials during their processing, and further maintaining the microbiological stability of raw materials for one month of storage. Moreover, the decontamination effect was maintained throughout the storage period, i.e. the surface of meat and fish was almost sterile for 30 days.

4. During storage of meat and fish raw materials in antiseptic ice of plasma-chemically activated aqueous solutions, slower accumulation of amino-

ammonia nitrogen is recorded (with the decrease 2 times for beef, 3 times for pork, 4 times for fish and chicken). Therefore, the use of the proposed antiseptic ice during storage of raw materials under study can partially prevent the protein breakdown and, accordingly, increase the shelf life of all considered raw materials to 20–30 days on average, since during this period it is possible to significantly slow down the processes of protein breakdown.

5. The paper proves the viability of use of antiseptic ice of plasma-chemically activated aqueous solutions during storage of meat and fish raw materials, since the obtained results indicate a pronounced disinfecting action of ice under study with peroxide concentration of 600 mg/l towards the raw materials. In addition, long-term effect of antiseptic ice on the raw materials during 1 month of storage is recorded.

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ВИКОРИСТАННЯ АНТИСЕПТИЧНОГО ЛЬОДУ З ПЛАЗМОХІМІЧНО АКТИВОВАНИХ ВОДНИХ РОЗЧИНІВ ПРИ ЗБЕРІГАННІ ХАРЧОВОЇ СИРОВИНИ

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Анотація. Технологія зберігання сировини тваринного походження в антисептичному льоді стала об'єктом підвищеної уваги вчених та фахівців харчопереробної промисловості. У роботі наведено особливості плазмохімічної активації розчинів для виробництва антисептичного льоду та їхнє подальше використання при зберіганні м'яса і риби. У процесі такої обробки утворюються мікрочастки пероксиду водню, які при контакті з сировиною здатні утворити активний кисень, що дозволяє дезінфікувати поверхню сировини. Визначено концентрацію пероксиду водню в розчинах, які в подальшому використовувались для отримання антисептичного льоду. Дослідження особливостей використання антисептичного льоду з плазмохімічно активованих водних розчинів показало, що при використанні представленого антисептика органолептика сировини була незмінна. Відмічено пригнічення життєздатної мікрофлори, за рахунок антимікробної дії плазмохімічно активованих водних розчинів. Антисептичний лід сприяє тривалому знезараженню сировини. Так в зразках відмічається відсутність мікрофлори мезофільних аеробних і факультативно-анаеробних мікроорганізмів та бактерій групи кишкової палички, що дозволяє покращити якість харчової сировини, що зберігається. При концентрації пероксидів на рівні 600–700 мг/л відмічається повна відсутність патогенної мікрофлори у зразках м'яса і риби. При зберіганні в льоді м'ясної та рибної сировини протягом 30 діб патогенна мікрофлора не з'являється, що підтверджує тривалу дезінфікуючу дію антисептичного льоду. Під час тривалого зберігання м'ясної та рибної сировини відмічене більш повільне накопичення аміно-аміачного азоту. Використання плазмохімічно активованих водних розчинів для виробництва антисептичного льоду і зберігання в ньому м'ясної і рибної сировини дозволяє частково попередити процеси розкладання білків і, відповідно, збільшити строки зберігання продукту. У роботі висвітлено технологічні параметри процесу зберігання м'яса та риби в антисептичному льоді, які можуть бути використані при промисловому зберіганні сировини. Наведено технологічні рекомендації щодо використання льоду з плазмохімічно активованих водних розчинів в процесі зберігання сировини тваринного походження.

Ключові слова: плазмохімічна активація, водні розчини, перекис водню, антисептичний лід, м'ясо, риба.