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Provision of Microbiological Safety in The Food Industry Based on Special Technological Supporting Solutions

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ABSTRACT

The present work has proved the effective application of the electrochemically treated solutions of sodium chloride (anolyte) as a technological auxiliary and disinfectant agent for processing agricultural vegetable plants - white cabbage (Brassica oleracia L.) and leaf lettuce (Lactuca sativa L.). The following solutions have a high antimicrobial activity against all types and forms of microorganisms, do not cause adaptive reactions of microorganisms, are innocuous to multicellular organisms with an immune system, and become an ordinary fresh drinking water under the degradation. To analyze the antimicrobial effect of the agent, microbiological studies of two kinds of test specimens of salad and vegetable products were carried out. The tests were carried out under the conditions of modeling the process of contact treatment with solutions of anolyte at a concentration of active chlorine of 460, 100, 50 and 10 mg /l and solutions based on sodium hypochlorite with an active chlorine concentration of 125 mg /l. Similar types of products without treatment with chlorine-containing solutions or water were used as control samples.

The prospects of the antibacterial action of the electrochemically activated disinfectant agents were judged by the microbiological indicators of the total viable count, bacteria of the family Enterobacteriaceae, including bacterium of intestinal bacillus (Coliform bacterias), and also by organoleptic indicators of the analyzed product quality. The obtained results of industrially manufactured white cabbage and leaf lettuce treatment confirm the effective usage of anolyte for decontamination of these products. The least effective regime is the treatment with a solution based on sodium hypochlorite, the confluent growth of microorganisms was observed under inoculation on the 14th day of store, at a time when the inoculation of the specimens treated with anolyte solution with an active chlorine concentration of 460 mg / l did not exceed the norm more than $1x10^4$ CFU/g. At the same time, the usage of anolyte solutions with an active chlorine concentration of 50 mg / l and 10 mg / l for 10 and then 5 minutes, respectively, contributes not only to a more effective reduction of total bacterial number compared to traditional treatment with hypochlorites, but also to achieving the necessary organoleptic aspects.

A comparative study of the electrochemically activated solution of chlorinated and hydroperoxide Russian-manufactured compounds (hazard category 4, the "low-hazardous substances" - the safest) with disinfectant Belgian-manufactured solution based on sodium hypochlorite (hazard category 3, moderately hazardous substances) established that the application of anolyte in the recommended regimes is more effective, leads to a decrease of the total viable count to 99.9%, and does not require rinsing with water cutting expenditure. Practical application of anolyte allowed to extend the shelf life expiration of salad products for 4 days. The established concentration of the solution in working and the found effective treatment regime provide microbiological safety and do not lead to a decrease of organoleptic quality aspects. The economic feasibility of the usage of developed regime at the food industry enterprise may be due to the cost reduction up to 20%.

Keywords: *green technologies, white cabbage, leaf lettuce, sodium hypochlorite, anolyte, microbiological safety, antimicrobial treatment*

INTRODUCTION

One of the priorities in the domain of food industry consists to increase the shelf life expiration of food products, taking into account the insuring of measures of consumer appeal. In the conditions of world population growth, the demand for food products also increases. In this regard, the processes of increasing of intensity and volumes of industrial production in the plant and animal industry inevitably occur, which create both new opportunities and new threats of obtaining potentially dangerous products.

Vegetable production occupies an important place in the daily ration of the people. As it is known, vegetables are an indispensable sources of water-soluble vitamins and a number of macro- and microelements. Despite this, the total level of consumption of vegetables in Russia is 100-110 kg, which is significantly lower than in most European countries.

One of the directions to increase the consumption of vegetables is the industrial production of freshly cut products, including salads on its base, its retail sale, as well as in the public food service establishments. However, the production of such products is associated with the risk of contamination by microorganisms both at the stage of processing, packaging and storage, with the loss risk of nutrients, especially of vitamins [5, 9, 17].

At the present time, the safety of fresh vegetables is achieved by producers mainly through the usage of chemical disinfection and preserving agents, which causes a negative attitude and distrust of consumers in this product, and also increases its cost. In view of the above said, it is promising to search for and implement less expensive technological solutions that, in terms of efficiency, will be highly competitive with the traditional methods of disinfection, in accordance with the standards of "green chemistry" and, as a result, will contribute to improving the safety of food products [1, 8, 16].

As domestic [1-13, 16] and foreign [14-19] experiences show, some of the most effective and environmentally friendly processing methods of raw materials and equipment in the food industry, taking into account the provision of microbiological safety and sanitary and hygienic control at food industry enterprises are the physical-chemical methods. It is important to specifically mention the method of electrochemical activation (ECA) of aqueous solutions. ECA oxidant solutions are highly effective disinfectant and detergent solutions characterized by a mixture of chlorine-oxygen and hydroperoxide compounds in a metastable condition - hypochlorous acid, chlorine dioxide, ozone, hypochlorite ion and other active forms of chlorine and oxygen [1-2, 10-11].

The oxidant solution can be used without individual protective equipment in presence of people, as it is classified as a minimum (IV) toxicity class; thanks to the metastable composition of the active substances, after the end of the disinfection treatment, it is degraded to non-hazardous substances and does not require further utilization; and it is not accumulated in the external environment.

At the present time, ECA solutions are used in a quite a number of branches of the national economy and industry. Thus, a special aqueous solution of anolyte is officially approved for using as disinfectant and sterilizing agents at public health facilities, for disinfection of premises and equipment at dairy, beverage processing, nonalcoholic and wine industries, for use as a technological intermediate agent to decontaminate vegetables in the food industry [16, 18].

The aim of the study is to develop technical and practical solutions for extending the shelf life expiration of food raw materials and food products of vegetable origin - salad products and white cabbage on the basis of the use of electrochemical processing methods. It is important to find work concentrations of electrochemically activated special aqueous solutions and treatment regimes that allow to control the quality and safety of plant raw materials.

MATERIALS AND METHODS

For assessing the antimicrobial effect of the electrochemically activated disinfectant agent, microbiological and organoleptic studies of the test specimens of two kinds of salad and vegetable products were made: white cabbage (Brassica oleracia L.) and leaf lettuce (Lactuca sativa L.) cultivar Afitsion, supplied and sold for the consumption in fresh form.

A solution of sodium chloride (anolyte, disinfectant agent, production: Russia [16]), obtained by electrolysis in a special electrochemical unit at a concentration of oxidants in active chlorine equivalents - 460, 100, 50 and 10 mg / l and pH - 5.0-6.5, was used to simulate the process of contact treatment of specimens. As a comparison of the effectiveness of the anolyte solution, a technological processing and disinfectant agent based on sodium hypochlorite (production: Belgium) was widely used in the food industry with a concentration of oxidants in active chlorine equivalents - 125 mg / l and a pH - 11.5.

Methods of treatment

For a comparative assessment of the impact of two types of agents, the following regimes of product processing treatment were used:

-treatment in the container with anolyte solution with an active chlorine concentration of 460 mg / l for 5 minutes;
-treatment with anolyte solution with an active chlorine concentration of 100 mg / l for 5 minutes;
-treatment with solutions of anolyte with an active chlorine concentration of 50 mg / l for 10 minutes followed by treatment in a second container with a solution with an active chlorine concentration of 10 mg / l for 5 minutes;
-treatment in a container with an aqueous solution based on sodium hypochlorite with an oxidant concentration in active chlorine equivalents of 125 mg / l for 5 minutes.

The specimens of the product without treatment with chlorine-containing solutions or water were used as the control. Under sterile conditions, the specimens weighing 10 g were placed in a 100 cm^3 container and added to each pre-staged disinfectant work solution in an amount that provided full immersion of the test specimens.

The effectiveness of decontamination of salad and vegetable products was assessed by microbiological indicators of the total viable count, bacteria of the family Enterobacteriaceae, including bacteria of intestinal bacillus.

Product samples for microbiological analysis were taken before the collection of specimens for sensory evaluation, using aseptic method, excluding microbial contamination of the product from the environment [1, 16].

Identification methods of microbiological contamination

To determine the total viable cell counts, those dilutions are chosen (not more than 1×10^4), cultivation of which being not less than 15 and no more than 300 colonies which will grow on the cup. In two Petri dishes, 1 cm^3 from each of the dilutions of the specimen was added. Then, 15-20 cm^3 of nutrient solution was added, after which the content of the Petri dish was immediately stirred to ensure the even distribution of the material and placed in a thermostat at 30-32°C for 72 hours. After thermostating, the number of grown colonies was counted. In view of the large number of colonies, the cups were divided into 2-6 identical sectors, then the number of colonies in 2-3 sectors was counted, but not less than 1/3 of the surface of the cup, the arithmetic mean of the colonies in one sector was found and then it was multiplied by the number of sectors in the cup.

The method of determining bacteria of intestinal bacillus is based on the inoculation of a certain amount of the product into a liquid selective medium containing lactose to determine fermentation capacity for the formation of acid and gas. The inoculation is carried out to the Kessler's medium with lactose in the dilution ratio of medium 1:10. In the analysis for the absence of Coliforms in 1 g of product, 10 cm^3 of a dilution product of 1:10 in 100 cc of Kessler's medium was inoculated. Test tubes with cultures were placed in a thermostat at a temperature of 34-36 °C for 24 hours, after which inoculation of Endo's medium was carried out for final conclusion about the presence of Coliforms in the product. The streak inoculation with a loop was performed on the surface of a well-dried, sector-separated medium to produce isolated colonies. Petri dishes with seedings were incubated at a temperature of 34-36°C for 24 hours. In the absence of colonies typical for the Coliforms, the sample is considered not contaminated [4, 7, 17].

RESULTS AND DISCUSSION

Microbiological examination of leaf lettuce

The experiment was carried out under the strict control of organoleptic parameters for 14 days, microbiological analyses were performed twice (in the first and 14th according to the normative documents, the expiration date of fresh salad products is determined by the manufacturer). The results of the study are clearly shown in Figures 1-4.

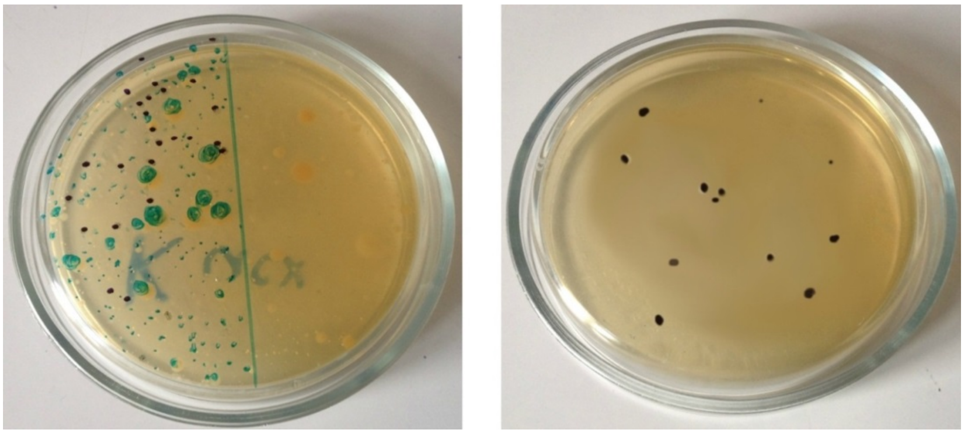
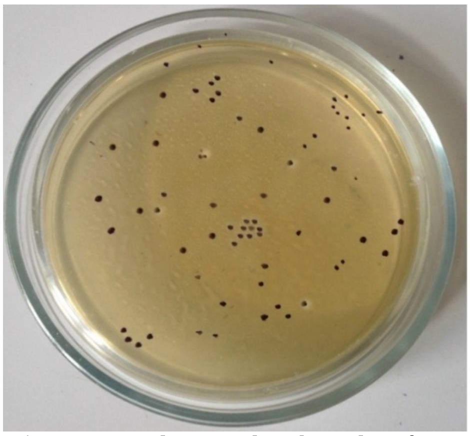


Fig 1. Control sample.
Without dilution before seeding

Fig 2. A sample treated with anolyte for5minutes. Without dilution



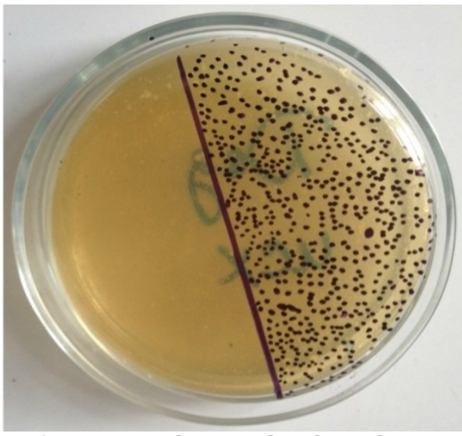


Fig 3. A sample treated with anolyte for 5 minutes, followed by washing with water. Without dilution

Fig 4. A sample treated witha sodium hypochlorite agent. Without dilution

The control specimen of the untreated salad (Figure 1) is totally contaminated, as evidenced by the large colonization. However, after the treatment with anolyte with an active chlorine concentration of 460 mg / l for 5 minutes (Figure 2), a significant decrease in the total bacterial count is observed. A sample treated with an anolyte solution with an active chlorine concentration of 460 mg / l for 5 minutes, followed by washing in a bath of distilled water (Figure 3), was half-seeded less than the control specimen. The treatment with an aqueous solution based on sodium hypochlorite with an active chlorine concentration of 125 mg / l for 5 minutes (Figure 4) was the least effective.

The control specimen without treatment with disinfectant agents and water washing (Figure 5) absolutely does not meet the safety requirements for salad products (according to TR TS, the amount of mesophilic aerobic and facultative anaerobic microorganisms, is not more than $1x10^4$ CFU/g in salad products). When inoculating the specimen treated with an anolyte with an active chlorine concentration of 460 mg / l for 5 minutes, a complete absence of colonies was observed in the dilution 10^4 (Figure 6).

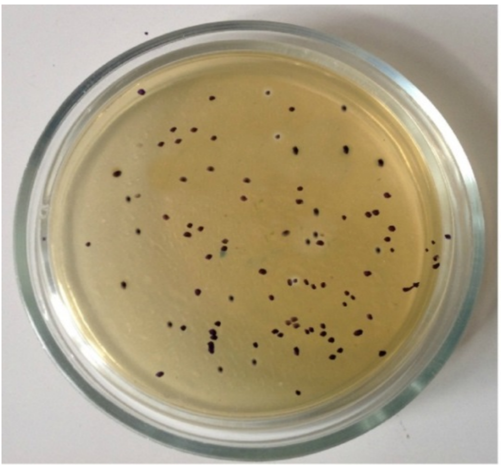


Fig 5. Control sample, with dilution 10^4 before seeding

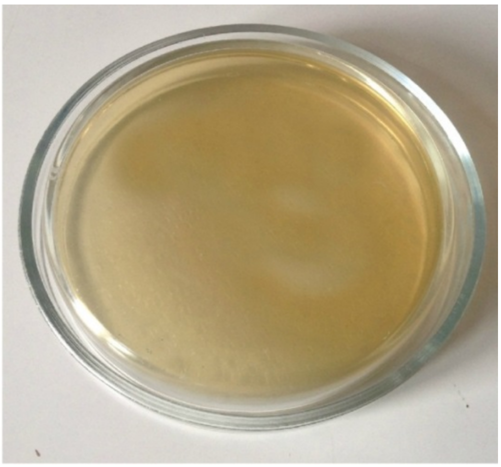


Fig 6. A sample treated with an anolyte for 5 minutes, with a dilution of 10^4 before seeding

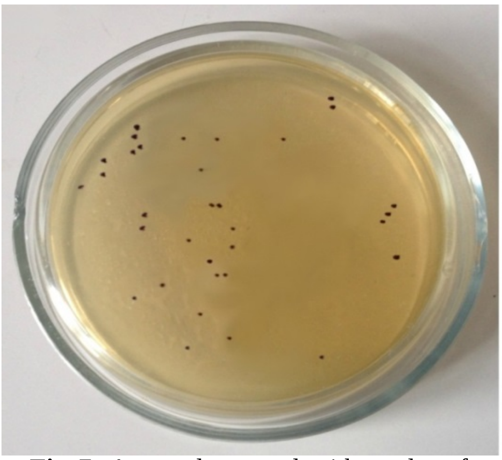


Fig 7. A sample treated with anolyte for 5 minutes, followed by water washing; with a dilution 10^4 before seeding

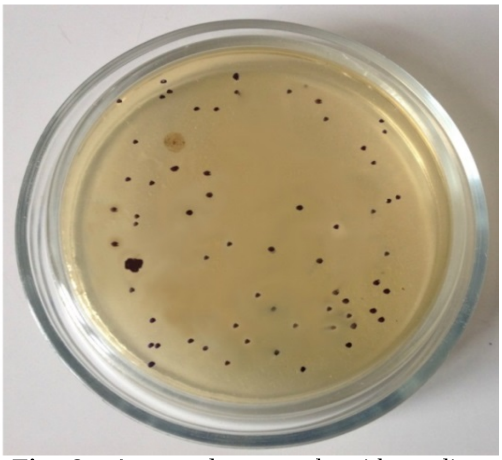


Fig 8. A sample treated with sodium hypochlorite based agent, with a dilution 10^4 before seeding

A sample obtained as a result of treatment with an anolyte solution with an active chlorine concentration of 460 mg / l for 5 minutes, followed by washing in a bath of distilled water (Figure 7), was diluted 10% with 55% less than the control specimen. The treatment with an aqueous solution based on sodium hypochlorite with an active chlorine concentration of 125 mg / l for 5 minutes (Figure 8) was not effective. The specimen is contaminated less than the control sample, but at the same time the total bacterial count exceeds the safety indicator.

As can be seen from the above, it may be deduced that the most effective treatment regime against the viable cell counts is a 5-minute immersion in an electrochemically activated aqueous solution with an active chlorine concentration of 460 mg / l.

Repeated microbiological examination of the specimens was carried out on the 14th day of storage. As a result, it was found that a significant decrease in the total bacterial count is observed under the treatment with anolyte solution with an active chlorine concentration of 460 mg / l for 5 minutes (50%). In addition, the reduction of colonies is noted in the sample treated with anolyte followed by washing with distilled water (by 30%). The least effective regime is the treatment with a solution based on sodium hypochlorite. When seeding on the 14th day of storage, a confluent growth of microorganisms was observed. The final data are summarized in Table 1.

Table 1. Indicators of the viable cell counts in CFU/g of lettuce in various treatment modes

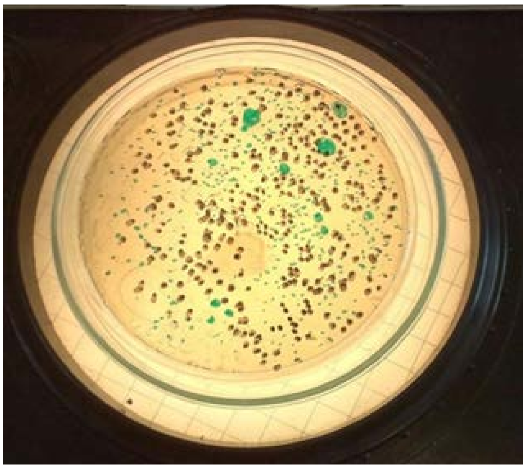
Treatment mode	CFU/g	
	Seeding after the treatment	Day 14 post treatment
Treatment with anolyte solution with an active chlorine concentration of 460 mg / l for 5 minutes	$1{,}2x10^3$	$5x10^3$

Treatment with anolyte solution with an active chlorine concentration of 460 mg / l for 5 minutes. Four-fold dilution	0	$1,5x10^4$
Treatment with anolyte solution with an active chlorine concentration of 460 mg / l for 5 minutes, followed by washing	4x10	confluent growth
Treatment with anolyte solution with active chlorine concentration of 460 mg / l for 5 minutes, followed by washing with water. Four-fold dilution	$3,5x10^3$	$1x10^2$
Treatment with a solution based on sodium hypochlorite with an active chlorine concentration of 125 mg / l for 5 minutes	$2x10^2$	confluent growth
Treatment with a solution based on sodium hypochlorite with an active chlorine concentration of 125 mg / l for 5 minutes. Four-fold dilution	$1,5x10^2$	confluent growth
Control sample without treatment	confluent growth	confluent growth
Control sample without treatment. Four-fold dilution	$5x10^2$	confluent growth

Analysis results for Coliform bacteria: in the used salad samples, the bacteria of intestinal bacillus were not detected. Organoleptic analysis showed that an unusual chlorine smell of the salad is the effect of 5-minute treatment. When processing the salad with anolyte solution with an active chlorine concentration of 460 mg / l for 5 minutes, followed by washing with water, the smell disappears. The product based on sodium hypochlorite did not affect the organoleptic properties of the product. It is important to note that after the treatment with anolyte, the color of the salad remained unchanged.

Microbiological analysis of white cabbage

The present experiment was based on the use of data obtained in the previous experiment. It was found that the best time for keeping the lettuce under the immersion in the electrochemically activated aqueous solution is 5 minutes. The disadvantage of the regime is the acquired smell of chlorine. Repeated washing with distilled water, as a factor that positively affects this indicator, adversely affected the purity of the sample treated with the anolyte. Consequently, it was decided to use the additional regimes with a decrease in the concentration of the solution and a ten-minute treatment of the product with anolyte with different concentrations of active chlorine. The results of the study are clearly shown in Figures 9-16.



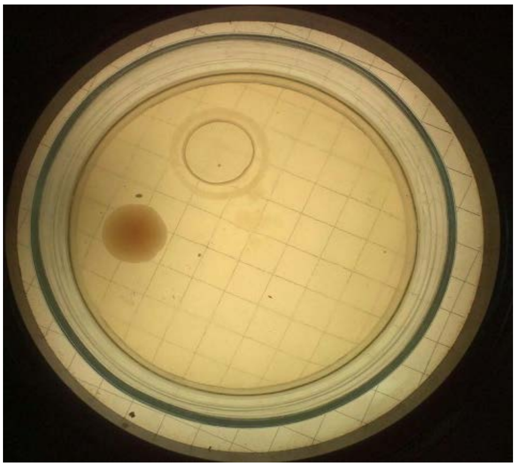
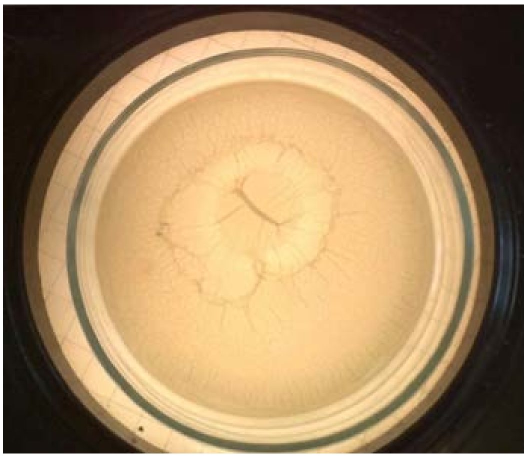


Fig 9. Control sample, seeding without dilution

Fig10. A sample treated with an anolyte at a concentration of 50 mg/l for 10 minutes; the second stage - anolyte with a concentration of 10 mg/l 5 min; seeding without dilution



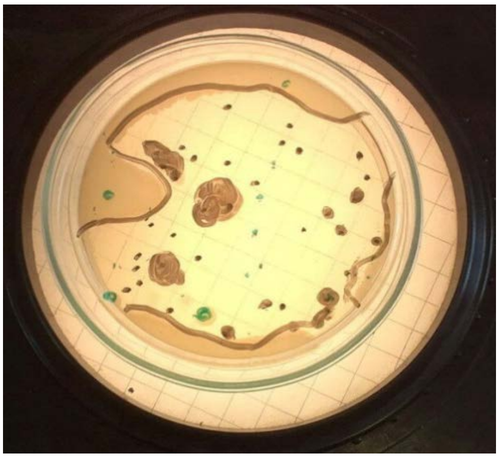
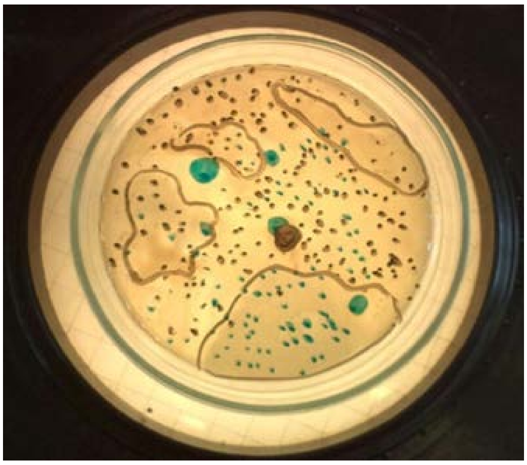


Fig 11. A sample treated with an anolyte at a concentration of 100 mg/l for 5 minutes; seeding without dilution

Fig 12. Specimen treated with a solution based on sodium hypochlorite concentration 125 mg/l 5 min; seeding without dilution



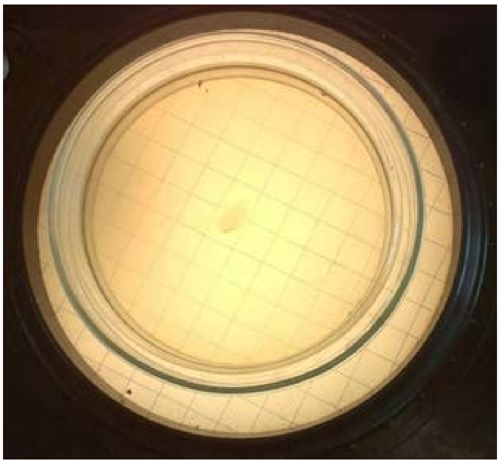


Fig 13. Control Specimen; dilution 10^4

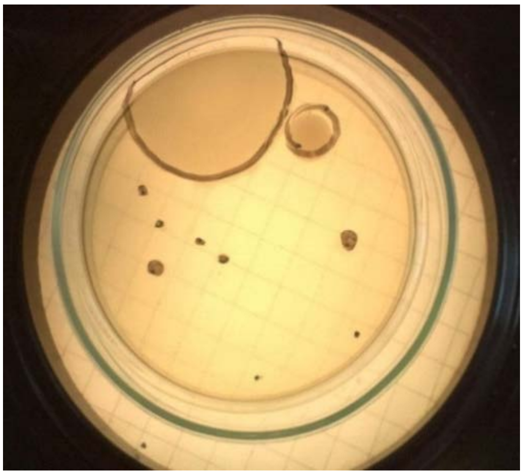


Fig 14. Specimen treated with an anolyte at a concentration of 50 mg/l 10min; the second stage - with a concentration of 10 mg/l 5 min; dilution 10^4

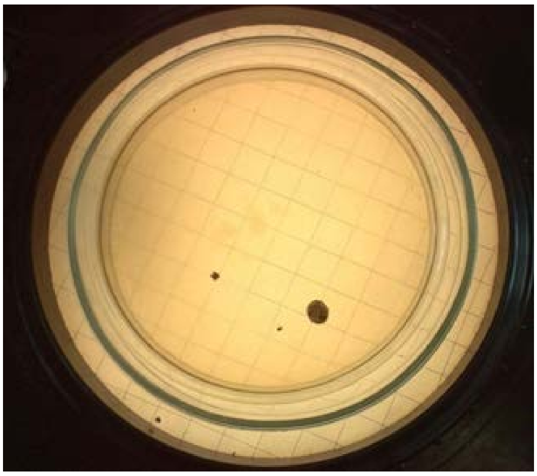


Fig 15. Specimen treated with anolyte at a concentration of 100 mg / l for 10 minutes; dilution 10^4

Fig16. Specimen treated with a solution based on sodium hypochlorite with a concentration of 125 mg / l 5 min; dilution 10^4

In the control specimen, a confluent growth of the colonies was observed in the initial concentration, which indicates an unacceptable contamination of the product (Figure 9). A large number of colonies are also present in a four-fold dilution (Figure 13). Treatment with an aqueous solution based on sodium hypochlorite with an active chlorine concentration of 125 mg / l for 5 minutes (Figures 12, 16) proved to be less effective, the specimen is contaminated less than the control specimen, but at the same time the total bacterial count exceeds the safety indicator. After the cabbage treatment with an anolyte solution with an active chlorine concentration of 50 mg / l for 10 minutes and then immersing it in an anolyte solution at a concentration of 10 mg / l for 5 minutes (Figure 10), a significant decrease of the total bacterial count is noted, and a full absence of colonies is registered in the dilution 10^4 (Figure 14). Treatment with anolyte solution with an active chlorine concentration of 100 mg / l for 5 minutes (Figure 11) allowed to significantly reduce the total bacterial count. A small number of colonies is present in the 10^4 dilution (Figure 15). The final results of the study are summarized in Table 2.

Table 2. Indicators of the viable cell counts of cabbage under different treatment modes

Treatment mode	Treatment time	CFU/g

Anolyte with active chlorine concentration of 100 mg / l	5 minutes	$3x10^4$
The first stage is an anolyte with an active chlorine concentration of 50 mg / l; the second stage - anolyte with an active chlorine concentration of 10 mg / l	10 minutes; 5 minutes	0
A solution based on sodium hypochlorite with an active chlorine concentration of 125 mg / l	5 minutes	$1,1x10^3$
Control specimen, without treatment and water washing	0 minutes	confluent growth

Coliforms in the samples of white cabbage were not detected. Sensors methods based on the analysis of sensations of the human senses are widely used to assess the consumer values of food products. For objective evaluation of the specimens, an expert group of 7 people was created and the sensors methods were carried out.
The statistical data are summarized in Table 3.

Table 3. Organoleptic evaluation of cabbage specimens after treatment

Mode treatment	Odor	Color	Taste
Anolyte 100 mg / l, 5 minutes	strong odor of chlorine	typical of the specimen	peculiar to the cabbage
Two-step treatment: 1. Anolyte 50 mg / l, 10 minutes; 2. Anolyte 10 mg / l, 5 minutes	peculiar to the specimen	more pale than the control specimen	
A solution based on sodium hypochlorite with an active chlorine concentration of 125 mg / l, 5 minutes	low odor of chlorine	typical of the specimen	peculiar to the cabbage
Control specimen, without treatment and water washing	peculiar to the specimen	typical of the specimen	peculiar to the cabbage

Due to the fact that the solutions studied are made on the basis of chlorine, one of the main criteria for evaluation of the processed foods is the absence of the acquired smell. The organoleptic analysis showed that after the two-step treatment of white cabbage (the first stage - anolyte with active chlorine concentration of 50 mg / l for 10 minutes, the second stage of anolyte with active chlorine concentration of 10 mg / l for 5 minutes), the smell of the samples did not change, the taste remained peculiar to the product. Treatment with an anolyte with a concentration of 100 mg / l adversely affected the aromatic properties of the sample, there was a strong odor of chlorine.
Based on the above, it can be concluded that the most effective treatment mode to reduce the total bacterial count is a two-step treatment with an anolyte with an active chlorine concentration of 50 mg / l for 10 minutes, followed by treatment in a second container with a solution with an active chlorine concentration of 10 mg / l in for 5 minutes.

An anolyte with a concentration of 100 mg / l negatively influenced the organoleptic properties of the cabbage sample, namely, changed the smell of the product, which was unacceptable, whereas the two-step treatment did not affect this indicator. A solution based on sodium hypochlorite, according to the results of the experiment, is the least effective of the analyzed agents. With an organoleptic evaluation of the cabbage treated with this disinfectant agent, the color change of the sample and the presence of a mild chlorine odor were established.

The performed experiments with scale-up confirmed the possibility of using special electrochemically activated solutions in the food industry to ensure microbiological safety. Practical application of anolyte allowed to extend the store terms of salad products for 4 days.

CONCLUSION

In this study, the effectiveness of electrochemically activated aqueous solutions of sodium chloride (anolyte) in the processing of raw materials of plant origin, in particular salad products and white cabbage, was proved.

The ways of technology improvement using this method were analyzed and identified. As a result of the conducted experiments, the following conclusions were made.

The most effective treatment mode to reduce the total bacterial count is a two-stage treatment with anolyte solutions with an active chlorine concentration of 50 mg / l for 10 minutes, followed by treatment in a second container with a solution with an active chlorine concentration of 10 mg / l for 5 minutes.

The absence of the negative effect of the developed solution of two-step treatment with a low concentration solution on the organoleptic properties of the quality of salad and vegetable products is shown. It was proved that at the specified processing, there is no change of quality, appearance, color. The odor of chlorine in the processed products was absent.

As a result of the comparison of the efficiency of salad and cabbage treatment with anolyte and sodium hypochlorite under different exposure regimes, it was found that the most effective antimicrobial agent was based on the electrochemical activation of an aqueous solution of sodium chloride. The insufficient effectiveness of a solution based on sodium hypochlorite is shown.

Thus, the technology of processing vegetable and salad products using electrochemically activated solutions as a disinfectant agent allows to exclude chemical preparations and suppress the development of bacterial diseases of sample plants.

In the course of the comparative analysis of the electrochemically activated solution of chlorinated and hydroperoxide compounds of Russian production (the technological processing and disinfectant Anolyte ANK SUPER, hazard category 4, the "low-hazardous substances - the safest) with disinfectant solution based on sodium hypochlorite (hazard category 3, "moderately hazardous substances") of Belgian production established that the use of anolyte in the recommended regimes is more effective, leads to a decrease of the total viable count to 99.9%, and does not require rinsing with water cutting expenditure. Practical application of anolyte allowed extending the shelf life expiration of the salad products for 4 days.

The established working concentration of the solution and the found effective treatment mode provide microbiological safety and do not lead to a decrease in organoleptic quality indicators. The economic feasibility of using the developed regime in a food industry enterprise can be caused by a cost reduction of up to 20%.

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